THE ACTINOMYCETES

VOLUME 2

CLASSIFICATION, IDENTIFICATION AND DESCRIPTIONS OF GENERA AND SPECIES

Selman A. Waksman

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Ferdinand Cohn (1828–1898), who was the first to observe and describe an actinomycete (1875), under the name *Streptothrix Foersteri*.

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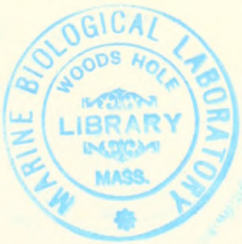

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PREFACE

In 1922, Professor D. H. Bergey of the University of Pennsylvania wrote to me that he and the Committee on Characterization and Classification of the Society of American Bacteriologists were in the process of preparing a "Manual of Determinative Bacteriology"; he asked whether I would be willing to undertake the preparation for that volume of a section dealing with the actinomycetes.

This group of organisms had occupied my attention for the previous several years, and alone (1919) and with Roland Curtis (1916), I had described a number of new species; yet I hesitated to accept this assignment. There were several important reasons for this hesitation: (a) I was not at all sure that the descriptions of actinomycetes so far published provided sufficient information for the accurate identification of most of the species recorded in the literature; (b) only four years previously, I had been warned by the dean of American cryptogamic botanists, Roland Thaxter, not to make further descriptions of new species based solely or largely upon cultural and biochemical properties; and finally (c) I was not even certain at that time whether the actinomycetes should be included with the bacteria.

I told Professor Bergey all this and suggested that it would be better to wait a few years until more detailed information was obtained concerning this group of microorganisms, especially with regard to their morphological and biochemical properties, before an attempt was made to codify them. I received a curt and somewhat sarcastic reply that if I would not, for one reason or another, undertake this task, he would have to do it himself. My immediate answer was, "I will do it." The best that I could accomplish at that time was to use cultural and biochemical characteristics as a major basis for the classification of the actinomycetes and for the characterization of the known species.

Since then, or for more than a third of a century and for seven consecutive editions of "Bergey's Manual," I have been largely responsible for the preparation of the descriptions of the actinomycetes. I have not, however, always had the final word in organization of the material for all the various editions. Alone (1940), and together with Professor A. T. Henrici of the University of Minnesota (1943), I proposed two systems for classification of the actinomycetes, the second of which consisted of a thorough revision of the group and its separation into four genera. The most significant change in this revision was the proposal, in 1943, of the new generic name, *Streptomyces*. This second system has been the basis for the organization of the material in the last two editions of Bergey's Manual.

In presenting this volume, I am now certain of one thing, namely, that the place of the actinomycetes is definitely among the bacteria and not among the fungi. Ample evidence of this belief has been presented in Volume I of this treatise. Unfortunately, the first reason for my hesitancy in 1922, I believe, remains valid; the accuracy of the information available for species identification is still

open to question. The chief reason for this uncertainty is that although much knowledge has since accumulated, especially during the last 20 years when many *Streptomyces* species became known as antibiotic-producing organisms, taxonomic work was largely neglected except by a few dedicated investigators. Recently, however, several important contributions (Hesseltine *et al.*, 1954; Flaig and Kutzner, 1954; Kutzner, 1956; Waksman, 1957; Ettlinger *et al.*, 1958; Pridham, 1959) to this subject have appeared. A survey of the recent literature shows that morphological characters are tending to replace physiological and cultural properties as the leading criteria in species characterization. It may be said that we are now in a transitional stage in which our ideas are changing, not only concerning the usefulness of criteria for species differentiation, but also with regard to the species concept. Since a classification of a group of living organisms is always only "preliminary," based upon the current knowledge of these organisms, I believe that, in summarizing the subject at present, and in trying to combine the older and newer ideas, I have presented useful criteria for species differentiation and an outline of species concept for the genera *Actinomyces*, *Nocardia*, *Streptomyces*, *Micromonospora*, and certain others.

The rapidly accumulating information about the separation of some of the genera into distinct groups or sections, the recent introduction of several new genera, and the description of numerous new species, all necessitated a complete recasting of the material presented in the last edition of Bergey's Manual and in other treatises. This volume is largely the result. An attempt has been made to bring together in this volume all the information required for the identification of newly isolated cultures of actinomycetes. All descriptions and names for which insufficient data have been provided, especially when no reproducible media have been employed, have been placed in a separate chapter as "incompletely described." Descriptions in which excessive and often confusing information has been presented, have been abbreviated to fit a certain "standard." Often, this standard has turned out to be a Procrustean bed. I beg forgiveness, both from the "reader" and from the preservers of the Code (International Code of Nomenclature of Bacteria and Viruses). My sole apology is that it is my sincere hope that it would serve the purpose.

The author wishes to acknowledge his sincere indebtedness to Dr. Norvel M. McClung of the University of Georgia, to Dr. R. E. Buchanan of Iowa State University, and to Dr. Ruth E. Gordon and Dr. Hubert A. Lechevalier of this Institute, for reading individual chapters and for making valuable suggestions; to Dr. Hans J. Kutzner of this Institute and Dr. Thomas G. Pridham of the Northern Regional Research Laboratory, for reading the major portions of this volume and for suggesting numerous corrections and modifications; to Miss Alma Dietz of the Upjohn Company, Dr. Edward J. Backus of the Lederle Laboratories, and all others who kindly supplied photographs; to Mrs. Herminie B. Kitchen for editorial work, and to Mr. Robert A. Day for assistance in the preparation of the various illustrations and for reading the entire manuscript,

Selman A. Waksman

INTRODUCTORY

This volume deals exclusively with the well recognized genera of the actinomycetes. No consideration is given here to the various closely related genera that are often included in the order *Actinomycetales*, notably the genus *Mycobacterium* Lehmann and Neumann, 1896.

The actinomycetes comprise three families, which are further subdivided into 10 genera.

A. Spores formed, but not in sporangia.
  I. Vegetative mycelium fragmenting into bacillary or coccoid elements.
    Family I. *Actinomycetaceae* Buchanan.
    1. Anaerobic or microaerophilic, nonacid-fast.
      1. *Actinomyces* Harz
    2. Aerobic, partially acid-fast or nonacid-fast.
      2. *Nocardia* Trevisan
  II. Vegetative mycelium nonseptate, not fragmenting into bacillary or coccoid elements.
    Family II. *Streptomycetaceae* Waksman and Henrici.
    1. Aerial mycelium produced.
      a. Spores formed in chains.
        3. *Streptomyces* Waksman and Henrici
      b. Spores formed singly.
        4. *Thermoactinomyces* Tsiklinsky
      c. Spores occurring in pairs or in chains.
        a^1. Mesophilic forms, in pairs.
          5. *Waksmania* Lechevalier and Lechevalier
        b^1. Thermophilic forms, in pairs or in chains.
          6. *Thermopolyspora* Henssen
    2. Aerial mycelium not produced.
      a. Spores occurring singly on short sporophores.
        a^1. Mesophilic forms.
          7. *Micromonospora* Ørskov
        b^1. Thermophilic forms.
          8. *Thermomonospora* Henssen
B. Spores occurring in sporangia.
  Family III. *Actinoplanaceae* Couch
  I. Aerial mycelium usually not formed, coiled conidial chains lacking, sporangiospores motile.
    9. *Actinoplanes* Couch
  II. Aerial mycelium abundant, coiled conidial chains as well as sporangia formed in some species, sporangiospores nonmotile.
    10. *Streptosporangium* Couch

Certain other genera, recently suggested, have been given tentative consideration.

These genera comprise about 350 species. In addition to these, a large number of other species are listed as "incompletely described."

TABLE OF CONTENTS

The Actinomycetes

VOLUME II

CLASSIFICATION, IDENTIFICATION, AND DESCRIPTION OF GENERA AND SPECIES

Chapter 1

The Species Concept in Relation to the Actinomycetes

Systematic Position of the Actinomycetes

In the preface to this volume, the statement was made that "I am now certain of one thing, namely, that the place of the actinomycetes is definitely among the bacteria and not among the fungi. Ample evidence of this belief has been presented in Volume I of this treatise." Nevertheless, some reiteration is warranted at this point.

The taxonomic position of the actinomycetes, notably their relationship to the bacteria, on the one hand, and to the fungi, on the other, has been one of the most debatable questions in microbiology. The size (width of thallus) and staining properties of the actinomycetes have usually placed them with the bacteria. Their branching and manner of sporulation have suggested their relationship to the fungi. Still other properties of actinomycetes seemed to warrant their consideration as a transition group between the bacteria and the fungi.

Recent evidence seems to point definitely to the fact that the actinomycetes are more closely related to the bacteria:

1. Some of the actinomycetes, such as species of *Actinomyces* and *Nocardia*, are closely related to true bacteria, notably species of *Lactobacillus* and *Corynebacterium*.

2. Neither actinomycetes nor bacteria have been shown to contain true nuclei; they both contain only chromatin granules distributed through the hyphae or the cells.

3. The diameter of actinomycete mycelium and spores is similar to that of bacteria. Actinomycetes also, as a rule, lack septa.

4. Actinomycetes are subject to attack by phages just as bacteria are; filamentous fungi are not.

5. Actinomycetes are usually sensitive (allowing for strain variability) to antibiotics that are active upon bacteria; they are usually resistant to those antibiotics, like the polyenes, that are active upon fungi but not upon bacteria.

6. Chitin is absent from the cell substance of actinomycetes as well as from bacterial cells, but is present in fungus mycelium and spores. In their lack of cellulose, actinomycetes are also similar to most bacteria and unlike fungi. Avery and Blank (1954) concluded that "from the chemical point of view *Actinomycetales* have nothing in common with the true fungi, but rather with the bacteria." Cummins and Harris (1958) went even further by suggesting that the order *Actinomycetales* be abolished altogether and that the families of the actinomycetes be included in the *Eubacteriales*.

7. Like bacteria, but unlike most fungi, actinomycetes as a rule are sensitive to an acid reaction of the medium.

8. The close relationship of the actinomycetes to the bacteria is also evident from the work of Couch (1954), who found that certain *Micromonospora*-like forms resemble

those of bacteria. Couch emphasized the resemblance of the mycelium and sporangia of *Actinoplanes* to those of the chytrids; he concluded that this genus may represent a connecting link between the bacteria and the lower fungi.

The Generic Problem with Actinomycetes

Prior to 1943, several systems of classification of actinomycetes had been proposed. In most instances, all the species were included in a single genus, which was frequently designated by different names. The most common of these names were the two oldest, *Streptothrix* and *Actinomyces*. Although occasional efforts had been made to separate the actinomycetes into several genera, such attempts usually failed to receive more than passing attention. The work of Waksman (1919), Ørskov (1923), Jensen (1931), and Erikson (1935) finally led Waksman and Henrici to suggest, in 1943, the division of the actinomycetes into four genera. A new genus, *Streptomyces*, was proposed to include those forms that are characterized by the production of an aerial mycelium with catenulate spores. Most of the important antibiotic-producing organisms subsequently have been found to belong to this genus.

Unfortunately, this generic separation brought with it a number of new problems, which can be briefly summarized as follows:

1. There is considerable overlapping among the different genera, notably between certain forms of *Streptomyces* that have lost the capacity to produce aerial mycelium and species of *Nocardia*, as brought out in a recent paper by Gordon and Smith (1955); there is also overlapping between certain nocardiae and mycobacteria.

2. The formation by species of *Streptomyces* and by certain forms of *Nocardia* of two different types of mycelium, substrate and aerial, and the influence of previous conditions of cultivation upon the growth and biochemical activities of these organisms served to confound the existing confusion.

The nomenclatural status of the genera of *Actinomycetales* has recently been discussed by Lessel (1960).

Lechevalier *et al.* (1961) described a new genus *Micropolyspora* (type species *M. brevicatena*), an organism that fragments like the members of the family *Actinomycetaceae* and sporulates like a member of the *Streptomycetaceae*, by forming chains of conidia on aerial hyphae; it also forms chains of conidia on the substrate mycelium. These authors suggested that the family *Streptomycetaceae* be dropped and the family *Actinomycetaceae* be enlarged to include the genera *Actinomyces*, *Micromonospora*, *Thermoactinomyces*, *Waksmania*, *Micropolyspora*, *Nocardia*, and *Streptomyces*.

What Is a Microbial Species?

In the study of the taxonomy of any group of living organisms, including microorganisms, one is faced sooner or later with the problem of defining what is meant by a species. With microorganisms, in usual practice, a microbial culture is designated by a name, sometimes qualified with a strain number; its morphological and cultural properties, and frequently its ecological and etiological characteristics, are described sufficiently so that anyone who finds this organism in nature will be able to recognize it from the description. If possible, the type form of the species is preserved in a type culture collection, to aid in the future identification of the species.

Unfortunately, microbial forms and types of organisms are not fixed in nature or even in culture. Some strains, even those closely related to the fixed type, may differ enough to raise a question as to their exact or specific identity. This frequently leads, often on the basis of only minor differences, to the creation of new species that are given new epithets. This is particularly true of those mic-

roorganisms, like the actinomycetes, that occur abundantly in nature; some of the newly isolated cultures may differ greatly from the fixed types. The difficulty of establishing and recognizing "species" under these conditions may become particularly perplexing. Raper (1954) was fully justified in saying, "It is almost axiomatic that the ease with which a species of microorganism can be recognized tends to vary inversely with the number of isolates available for observation and examination."

The concept of "species" first used during the seventeenth century gradually came to denote the fundamental units of a biological classification. These units came to be regarded as fixed or static entities, created by nature, which can be grouped into higher categories, namely, genera, orders, and classes. As the evolutionary theory was gradually accepted, especially with the development of modern genetics and cytology, the concept of "species" began to undergo a change.

Hucker and Pederson (1931) emphasized that the difficulty of dividing lower forms into well-defined species has led many to question whether these are natural groups and whether they can be considered to be similar to "species" among higher forms of life. The problem always arises: How much difference must exist between two cultures of bacteria before we are justified in regarding them as distinct species?

Krassilnikov (1938) was very emphatic in stating that many investigators, without considering the rules of nomenclature proposed at international congresses, either describe the same forms under different names or combine various organisms into the same species. He said: "Even the concept of 'species' is considered differently by various workers depending on their individual point of view, frequently considering a minor lack of correlation of a certain character as sufficient justification for creating a new species."

Just as in the case of many groups of true bacteria, one of the causes of the chaotic state of nomenclature of the actinomycetes is the lack of type cultures. It has actually been suggested (Skerman, 1949) that even the available cultures be completely redescribed, priorities being based on existing names, and those names and descriptions for which no type cultures are available be discarded.

In comparing the species concept among microbes with that of higher plants and animals, Cowan (1956) suggested that consideration be given to the following aspects: (a) whereas larger plants and animals have geographical distribution areas, few microbes have such particular areas; (b) morphology is essential for the separation of species among algae, fungi, and protozoa, but it barely distinguishes higher ranks among bacteria; (c) cytology is useful at the generic level, but "at the species level the bacteriologist relies more on physiological than on morphological differences"; (d) interfertility is hardly to be considered as a species character, since bacteria and actinomycetes reproduce asexually; (e) the introduction of certain characters in microbiology not utilized by botanists and zoologists adds satisfactory classification criteria; these include "nutritional requirements, metabolic and catabolic products, antigenic structure and pathogenicity."

In discussing bacterial classification, Sneath (1957) came to the following conclusions: (a) an ideal classification is one which has the greatest content of information; (b) over-all similarity is the basic concept of such an ideal classification, and is measured in terms of the number of similar features possessed by two organisms; (c) every feature should have equal weight; (d) the division into taxonomic groups is made upon correlated features.

To avoid the growing confusion from conflicting ideas, Gilmour (1958) suggested sep-

aration of the concepts of "nomenclatural taxonomy" from those of "experimental taxonomy." It is to be remembered that species are, after all, convenient "artificial creations of human imagination" rather than "real biological entities." Gilmour further suggested that "nomenclatural categories of genus, species, variety, etc." are excellently suited for the purpose of "a broad map of the diversity of living things." It would, therefore, be "a great advantage if they were not subject to continued attempts to bring them up to date and to redefine them in evolutionary terms."

Speciation of Actinomycetes Other than Streptomycetes

Krassilnikov (1938) wrote, "In spite of the most extensive literature, we have no definite idea concerning the natural systematics of the actinomycetes, nor a single opinion of their structure and development." The recently accumulated information leads us to conclude, however, that we need not be so pessimistic.

According to Pridham (1959), there are now known more than 100 genera of actinomycetes and well over 1500 subgeneric names and specific, or subspecific, epithets. Some of the descriptions of these forms are good, others lack essential details, and many are worthless. Morphological criteria are believed to play an important role in separation at the generic level (Fig. 1), with a gradual intergradation in complexity of reproductive units. The actinomycetes are looked upon as a heterogeneous group of organisms, ranging from the simple mycococci and the seemingly more complex nocardiae to the straight or flexuous streptomycetes and the verticillate forms, and from the relatively simple micromonosporae to forms such as *Waksmania*, *Actinoplanes*, and *Streptosporangium* (the latter two genera possibly having some affinities with the chytrids). Some of these organisms have definite affinities with true bacteria, others with both bacteria and microfungi, and still others with phycomycetous fungi.

This heterogeneity is further emphasized by the facts that the actinomycetes contain forms that are anaerobic, microaerophilic, or aerobic; forms that fragment and those that do not; and forms that produce aerial mycelium and those that do not. Pridham suggested that some of the present concepts centered around the three genera *Actinomyces*, *Nocardia*, and *Streptomyces* be accepted. Thus included in the *Actinomyces* would be the anaerobic to microaerophilic forms; in the *Nocardia*, the aerobic types that either form no aerial mycelium or produce an aerial mycelium that generally has no catenulate spores; and in the *Streptomyces*, the aerobic forms that generally produce catenulate spores.

Although time and again taxonomists have emphasized that an effective system of classification should be based upon criteria that are expressed in consistently reproducible results, this has hardly been applied, at least so far as our present knowledge is concerned, to the species characterization of actinomycetes. Many "new species" have been described on the basis of a single difference—frequently a quantitative variable—from "old species." One often wonders what the composition of the medium, the conditions of growth, and the natural variability observed so frequently among duplicate cultures have to do with these distinguishing properties.

The species concept among the actinomycetes must be considered as the continuity between different groups of organisms designated as species, with various transitional forms bridging the gaps between species. The concept of *natural classification* applies to actinomycetes perhaps better than to many other bacterial groups: there are the chemical approach (chemical composition, presence of specific chemical compounds), the morphological approach (type of aerial my-

FIGURE 1. Morphology of the various genera of the Actinomycetales. Of these, only *Actinomyces* in A, *Nocardia* in B, *Streptomyces* in C, *Micromonospora*, *Thermoactinomyces*, *Waksmania*, *Actinoplanes*, and *Streptosporangium* in D are recognized in this treatise as true actinomycetes; *Nocardia* and *Proactinomyces* are synonyms (Courtesy of T. G. Pridham of the Northern Regional Laboratory, Agriculture Research Service, U. S. Department of Agriculture).

celium, type of sporulation, shape and surface of spore), and finally the ecological approach (anaerobic *versus* aerobic, pathogenic *versus* nonpathogenic, thermophilic *versus* mesophilic). The idea of a *physiological classification* includes formation of antibiotics and of enzymes, utilization of carbon compounds, and transformation of nitrogenous compounds, all of which can supply supplementary information.

Speciation of Streptomycetes

What has been said for the actinomycetes as a whole applies particularly to the large, heterogeneous, and variable group of organisms represented in nature by the aerial mycelium-producing strains, most of which are included at present in the genus *Streptomyces*. These organisms are found in the soil in the form of hundreds of thousands of spores and of bits of mycelium per gram. They are also found extensively in manures and in composts, in various fresh-water basins, in dust, and on food. They are almost entirely absent from peat bogs and the sea.

The actinomycetes belonging to the genus *Streptomyces* have recently come to occupy an eminent place because many of them are important producers of antibiotics, vitamins, and enzymes.

With the growing economic significance of members of the genus, the establishment for each species of certain characteristics which would be adequate to enable the investigator to recognize freshly isolated cultures in well defined specific terms becomes of great theoretical and practical importance.

Following the first descriptions of Cohn (1875), very few additional species of the aerial mycelium-producing actinomycetes were recognized until 1914. This was true in spite of the rapidly accumulating literature on the occurrence of such actinomycetes in the soil and in the causation of plant diseases. The common designations were limited largely to the names "*Actinomyces albus*" and "*Actinomyces chromogenus*," depending on the color of the aerial mycelium or the formation of soluble, dark pigments in complex organic media.

Rossi-Doria (1891) was the first to describe an organism, under the name *Streptothrix alba*, which was later designated as the type of the genus *Streptomyces* proposed by Waksman and Henrici in 1943. The most important characteristics of this species are its white aerial mycelium and the tendency for colonies to form concentric rings of this aerial mycelium. Rossi-Doria noted the ability of his organisms to grow on numerous complex organic substrates.

Thaxter (1891), who first described an important economic species, the causative agent of potato scab (which he believed to be a fungus, *Oospora*), was highly critical of the efforts to describe "species" largely on the basis of cultural properties of the organisms. In this respect, the actinomycetes do not differ from any of the other groups of bacteria, where cultural properties and biochemical reactions have to supplement insufficient morphological information. Physiological activities and ecological properties, which are the expression of the response of organisms to their environment, are too numerous and often too variable among actinomycetes to justify unlimited confidence.

Krainsky (1914), Waksman and Curtis (1916), and Waksman (1919) emphasized the use of synthetic substrates, in addition to organic media. Carbon and nitrogen utilization tests were employed. Added attention was given to micromorphology. Many new species were described. Jensen (1930a, 1931) and Duché (1934) added various new species, the latter investigator stressing the use of various combinations of carbohydrates and nitrogenous compounds as media ingredients.

One of the reasons for the limited recognition of species among the aerial mycelium-producing actinomycetes prior to 1914 was the fact that protein-rich media were

employed for their cultivation. With the introduction of synthetic media, it became definitely established that the aerial mycelium-producing actinomycetes comprise a large number of forms, differing greatly in their physiological and biochemical properties, and to a lesser degree in their morphology. It was also recognized that, if a sufficiently large number of cultures was isolated and examined, many differences would be noted suggesting variability of the type species. The concept "species-groups," with one culture as the type species, was suggested. Waksman (1919) emphasized, therefore, that in spite of variation of individual biochemical characteristics of the actinomycetes, there are certain well defined properties, notably morphology, color of aerial mycelium, and formation of soluble pigments, that characterize these organisms, especially when grown on standard synthetic media and under carefully controlled conditions of temperature and aeration.

It is easy to pick out a few cultures of actinomycetes (or streptomycetes) which possess characteristic properties that can be recognized as distinct species, and to discard all the others. This was actually done by Waksman and Curtis in their early (1915–1916) classification of actinomycetes, since they were faced with such a large number of freshly isolated cultures that it was impossible to consider more than a very small number of them. How many others have acted likewise it is difficult to say. Should the various intermediate strains be considered, one might be inclined to regard each as a different species, distinct from the others in at least one variable property, be it morphological, cultural, or biochemical. With the examination, in recent years, of many thousands of cultures of actinomycetes for their antibiotic properties, such an attitude was frequently reduced to an absurdity. There are those who contend that the insistence on permanent characteristics, preferably a group of them, in describing new species, would limit greatly our recognition of the growing economic importance of these organisms. Then there are those who reason that not enough species of actinomycetes have so far been described, thus justifying random descriptions of many freshly isolated strains as new species.

Even synthetic media did not yield the final answer to the species problem of this group of organisms. Their cultural properties, or growth characteristics in media of different chemical composition, properties that were at first greatly emphasized, were found to be extremely variable. Type cultures were shown to change their specific characteristics when grown in artificial media. Saltations and mutations came to play a highly important part in changing such properties. When morphology was recognized at all, it was limited largely to observations on the curvature of the sporophores or to the size and shape of the spores. Drechsler (1919) was the first to make a detailed study of the morphology of the actinomycetes that produce aerial mycelium. Unfortunately, he limited his study to a small number of cultures; this prevented him from establishing the existence of many specific types which could have been recognized on the basis not only of cultural but also of morphological properties.

It must be regarded as a considerable step backward when Lieske (1921) completely disregarded the work of Krainsky (1914), Waksman and Curtis (1916), Conn (1917), and Waksman (1919). He believed that the classification of actinomycetes was impossible, since the properties observed were highly variable. His skeptical attitude toward the question of speciation of actinomycetes was due largely to his use of complex media for the growth of these organisms, and to a lack of sufficient appreciation of the significance of simple media for their characterization.

Burkholder *et al.* (1954) were led to conclude that the species concepts formulated by an individual investigator depend a great deal upon the investigator's personal experience, and whether he is a "splitter" or a "lumper." They suggested further that microbial species should be characterized by multiple, readily recognizable, and reasonably stable properties; the history of the cultures and the nature of the medium in which they are growing are of prime importance.

With the genus *Streptomyces* gaining considerable economic importance, the creation of many new species based upon biochemical properties, notably formation of antibiotics, resulted in much confusion in the recognition of some of the species. The use of various mutagenic agents, such as irradiation, led to the formation of new forms or strains which are often markedly different in their nutrient requirements and biochemical activities from the mother cultures.

According to this concept, in the classification of a group of living organisms, no single feature can be taken as the predominant character. Only when this is combined with a group of other characters is one able to separate the group into subgroups, notably genera and species. In selecting a character, no matter what its importance in the primary subdivision of a group of actinomycetes, one may begin with color; or structure of aerial mycelium; or certain biochemical reactions, which may comprise proteolytic activities, utilization of carbohydrates, production of antibiotics, or phage sensitivity. The important thing is to select a group of properties to characterize each species, with fewer characters, perhaps only one, such as antibiotic production, characterizing varieties. One always encounters, of course, the intermediate forms between the species. Each investigator will have to decide upon the basis of the combination of characters whether to place an unknown culture with one species or another. Thus the concept of species-group or section has come into being. As a further illustration one may take *S. griseus* and *S. griseinus*, two species belonging to the *S. griseus* group; both are nonchromogenic; the color of the aerial mycelium of both is similar; they are both similar morphologically; yet they are different from the standpoint of carbon utilization, phage sensitivity, and antibiotic production.

Flaig and Kutzner (1954), Kutzner (1956), Baldacci (1959), and numerous others emphasized both physiological and morphological criteria. Gause *et al.* (1957) emphasized the color of substrate and of aerial mycelium as well as morphology of sporulating hyphae. Numerous new species and varieties were described, although very few prior named species were discussed or placed into their system of classification. Many of these species and varieties are no doubt synonymous with previously described forms.

With streptomycetes, the species are linked together so gradually that it is very difficult to say where one species ends and another begins. The creation of "sections," "groups," or "series" to occupy an intermediate place between genera and species may help in clarifying relationships, but it does not do away entirely with the potential confusion in the creation of new species, especially when the relation of such species to those already established is not sufficiently understood. This confusion has led some investigators to question "whether the species concept is tenable in microbiology, and if it is not, what we are to substitute for it." It has even been suggested that the idea of static species must be abandoned in favor of something more elastic.

Even now, after many additional data have accumulated concerning the morphology of the actinomycetes, and after these organisms have been separated into a number of genera, there is still no general agreement concerning characterization of species.

Krassilnikov (1949) insisted that the shape of the spore, as seen in the light microscope, should be recognized as the major criterion for species differentiation. It is doubtful, however, whether Krassilnikov's various "longisporus" and "globisporus" types, with their many subtypes, can greatly facilitate the solution of the problem of species characterization. The cultural properties of these organisms still offer some of the most important criteria for species differentiation. There is also now available sufficient additional information concerning morphology, such as formation and branching of the sporophores, formation and nature of spores, and especially the spore surface as shown by the electron microscope, to make possible the use of these criteria not only for supplementary but often for major characterization of the species.

Several factors have thus contributed to the confusion in establishing and recognizing species of actinomycetes: (a) lack of clearly defined morphological characters; (b) great variability of these organisms; (c) occurrence of numerous transition types; (d) ease of formation of mutants; (e) lack of sufficiently recognizable type species; (f) lack of emphasis upon species-groups and upon type cultures; and (g) insufficient recognition of the formation of well-defined chemical compounds which could be used as additional criteria for species characterization.

The suggestion that closely related species be placed in "species-groups" or "aggregate-species" has recently been gaining considerable attention. Such a unit should be characterized by various reproducible properties under standard conditions of culture. Baldacci *et al.* (1953, 1956) suggested that micromorphological criteria, namely, segmentation and branching of vegetative mycelium, presence or absence of spores, and arrangement of sporophores, be used for generic classification. The genus *Streptomyces* was then divided, on the basis of pigmentation of the vegetative and aerial mycelium, into a number of "series," each of which was further subdivided into species. Gause *et al.* (1957) made use of the "series" concept and created a number of groups based on the pigmentation of the aerial mycelium.

When so many different cultures of actinomycetes can be isolated easily from natural substrates, it is but natural that various intermediate types should be found and that established species should tend to overlap one another. If one were to isolate only a small number of cultures, it would be simple to recognize a few well defined species. But when hundreds of similar strains are found in nature and when many of them show only minor variations from one another, variations which are not important enough to warrant creation of new species but are nevertheless variations from the established type, the difficulties mount rapidly (Fig. 2).

When study is based upon a single strain, a particular species may be described as having a yellow or yellowish aerial mycelium. Another strain may produce, on the same medium, an aerial mycelium only a shade different in color from the original type; this pigment may be designated as sulfur-yellow, cream-yellow, saffron-yellow, or even brownish, all other physiological and morphological properties being similar. Would one be justified in calling such a new strain a different species? The answer is definitely "no." One culture may produce a strong tyrosinase reaction, and another only a weak reaction, as indicated by pigmentation with potato, gelatin, and other protein media. One would be inclined to accept these as mere quantitative variations allowable for an established species. This must be recognized, since it is well known that had the test been repeated in another laboratory, where the medium might be slightly different in composition, the method of sterilization of the medium different, or the age and origin of the inoculum different, these variations

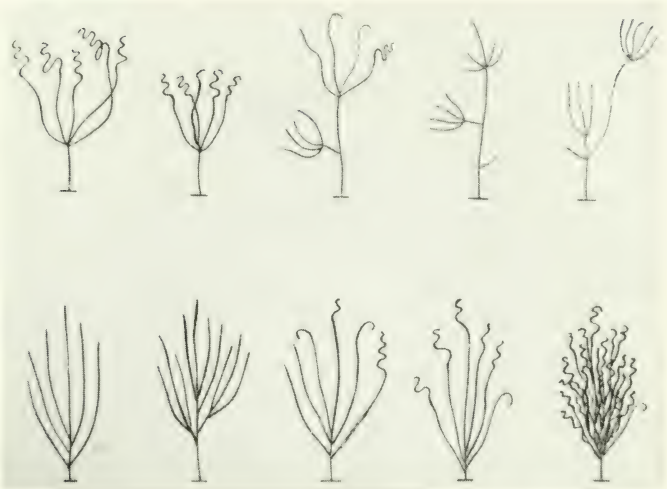

FIGURE 2. Schematic representation of tuft and cluster formation by certain *Streptomyces* species (Reproduced from: Shinobu, R. Mem. Osaka Univ. Lib. Arts and Ed. B. Nat. Sci. 7, 1958).

might have been sufficient to account for the minor differences in the color of mycelium or in the pigmentation of the medium. But what is one to do when the original culture is recorded as producing a yellow aerial mycelium on a given medium, whereas the new isolate gives a buff or brown mycelium? The answer would be that if all the other recognizable properties are the same or similar, this would be nothing more than a variant. Were one to plate out a single culture and pick a large number of colonies, similar variations could no doubt be observed.

Unfortunately, it has frequently been found much easier to assign undue importance to these variations and designate a freshly isolated culture as a new species. Some justification for this attitude has been found in the fact that the new culture may possess an important economic property, such as the production of a new antibiotic. It is largely for this reason that within the last 15 years more "new" species have been created than in all the previous 75 years since Ferdinand Cohn first described his *Streptothrix*.

Requirements for Adequate Species Descriptions

In accordance with the rules of the International Code of Nomenclature of Bacteria and Viruses, certain procedures must be followed in describing bacterial species. These are summarized by Ainsworth and Cowan (1954) as follows:

The name must be effectively published.

The name must be validated by a concise description of the diagnostic features of the new isolate.

The etymology of the name should be explained.

No Latin diagnosis is required.

When descriptions are reported in a language unfamiliar to the majority of workers, it is recommended that the authors simultaneously publish the diagnosis in a more familiar language.

Subcultures of the type strains should be deposited at one or more of the national culture collections.

Unfortunately, these simple rules have

not always been adhered to. Numerous names of actinomycetes are reported in the literature with no descriptions whatever. Some of the descriptions have been published partly in languages not generally accessible, or in the form of patents, or even as news announcements in trade or popular journals.

Although every effort has been made in this treatise to include all species that have been adequately described, numerous forms must be listed as "incompletely described" (Chapter 13). Various names are listed for which not even an inadequate description is available, the temptation to name a culture as a new organism, in order to claim the discovery, being too great.

Chapter 2

The Genus *Actinomyces*

The genus *Actinomyces* comprises anaerobic or microaerophilic organisms. They are mostly pathogenic in nature. The pathogenic forms are nonacid-fast, nonproteolytic, and nondiastatic. These have been isolated from granules in the pus of morbid tissues of a human and animal disease known as actinomycosis. They produce no filterable stages and show no serological reactions with other genera.

There are also on record observations concerning the occurrence in various natural substrates of nonpathogenic, mesophilic, anaerobic actinomycetes that can with full justification be included in this genus. Although few of these have been sufficiently studied, one such species is included. The saprophytic forms may be proteolytic, actively fermentative, and may possess marked reducing properties.

The natural relationship of this genus to the other genera of the actinomycetes, based primarily upon morphological and cytological studies, has recently been examined by Bisset (1959).

Classification of the Genus *Actinomyces*

I. Pathogenic forms or forms isolated from pathogenic specimens.
  1. Colonies soft, smooth, uniform, not adherent to the medium. No aerial hyphae.
    a. Causative agent of certain animal diseases.
      1. *Actinomyces bovis*
    b. Isolated from human saliva and carious teeth.
      8. *Actinomyces odontolyticus*
  2. Colonies tougher in texture and warted in appearance, adherent to medium. Aeria hyphae rare.
    a. Hyphae gram-positive and stain faintly with hemotoxylin. Causative agent of certain human diseases known as actinomycosis.
      6. *Actinomyces israelii*
    a[1]. Related form.
      4. *Actinomyces discofoliatus*
    b. Hyphae in pus granules stain with basic stains. Cause of actinomycosis in cats and dogs.
      2. *Actinomyces baudetii*

II. Nonpathogenic forms.
  1. Occurs in human mouth.
    7. *Actinomyces naeslundii*
    a. Related form.
      3. *Actinomyces cellulitis*
  2. Occurs in ground waters.
    5. *Actinomyces hvidhanseni*

According to Thompson (1950), there are two distinct species of anaerobic organisms that should be included in the genus *Actinomyces*: *A. bovis* which is responsible for most cases of lumpy jaw in cattle, and *A. israelii* which causes most of the typical infections in man. This separation of the genus agrees with the concepts of other investigators. One strain of *A. israelii* was recovered from a bovine source, and it was suggested that some bovine infections may be due to *A. israelii*. On the other hand, the work of Holm (1951) and Lentze (1948) indicates that a small number of human infections may be due to *A. bovis*.

Cummins and Harris (1958) fully supported the conclusions of Erikson (1940) and Thompson (1950) that bovine and human strains of *Actinomyces* are distinct. On

the basis of their chemical data, they suggested that there was very little justification for placing bovine strains even in the same genus with the strains of *A. israelii*. Of the 12 strains received as *A. bovis*, two were identical with the human strains, two showed a cell-wall pattern unlike anything hitherto recorded, two appeared to be corynebacteria, and the remaining six formed a homogeneous group which seemed to be closely related to lactobacilli. If cell-wall composition is to be considered as any guide to the classification of these strains, the criteria used for the identification of *A. bovis* are insufficient and many of the investigators who identified the strain were not properly qualified to do so.

Thompson and Lovestedt (1951) isolated cultures from the mouths of 24 patients. In addition to two positive cultures of *A. israelii*, nine of the cultures comprised an organism which grew under both aerobic and anaerobic conditions. They considered the latter to be a saprophyte found in the mouth, frequently confused with *A. israelii*. The name *A. naeslundii* was proposed for these cultures.

Howell *et al.* (1959) made a comparison of 200 strains of *Actinomyces* isolated from the oral cavity in the absence of actinomycosis, and 11 isolated from actinomycotic lesions. These strains were of two main types, one corresponding to the organisms described under the name *A. naeslundii*, and the other essentially identical to those isolated from lesions, which should be designated as *A. israelii*. They recommended that *A. naeslundii* Thompson and Lovestedt be accepted as the proper name for the rapidly growing facultative type of *Actinomyces*.

One may finally report the results of a comparative study (Pine *et al.*, 1960) of 11 bovine strains of *Actinomyces* isolated from typical cases of lumpy jaw and 15 human strains which had been identified as *A. israelii* and *A. naeslundii*. Of the bovine strains, one was a typical *A. israelii*, whereas the remaining strains formed a homogeneous group of fast growing, catalase-negative diphtheroids which invariably failed to form a true mycelium *in vitro*; they were thus different from both *A. israelii* and *A. naeslundii*. The last 10 strains comprised the classical *A. bovis*. They produced two kinds of colonies, depending on the medium: one smooth colony, identical to that of *Corynebacterium acnes*, and one rough similar to that of *A. israelii* but with no mycelium. They were anaerobes, forming acid from glucose but none from xylose, raffinose, or mannitol; nitrates were not reduced and starch was rapidly hydrolyzed. They were less pathogenic for animals than human strains, but induced lesions in which actinomycotic mycelial clumps were formed. The *A. israelii* strains were also anaerobes; they formed acid from glucose, usually from xylose and mannitol, and less often from raffinose; nitrates were sometimes reduced to nitrites, and starch was poorly hydrolyzed if at all. *A. naeslundii* strains were facultative anaerobes and formed acid from glucose and raffinose, but none from xylose or mannitol; nitrates were reduced to nitrites and starch was poorly hydrolyzed. Micromanipulative methods for the study of microaerophilic organisms have been examined by Erikson (1954); the catalase reaction of *A. bovis* was reported by Suter (1956).

According to Emmons,* there is little value in presenting as valid all the following species until they have been studied carefully in pure culture. He suggested to accept only *A. bovis*, *A. israelii*, *A. baudetii*, and *A. naeslundii*. He went so far as to suggest that the staining reactions of *A. baudetii* are hardly sufficient for its differentiation.

Descriptions of Species of *Actinomyces*

1. *Actinomyces bovis* Harz (Harz, C. O. *In* Bollinger, O. Centr. med. Wiss. **15:** 485,

* Personal communication.

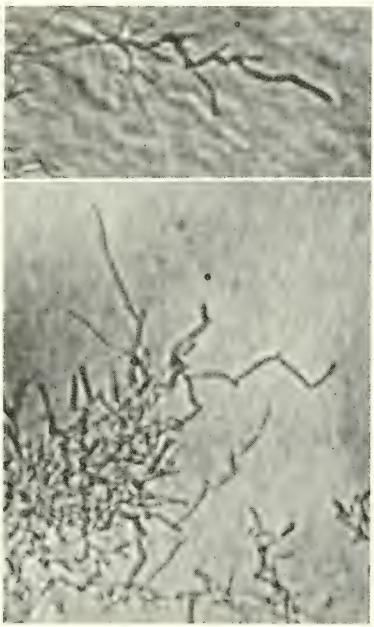

FIGURE 3. *A. bovis*, branching mycelium; cultured from human tonsils, × 1000 (Reproduced from: Emmons, C. W. Puerto Rico J. Public Health Trop. Med. **11**: 720, 1936).

1877; Jahr. Münch. Thierarzeneisch **5:** 125, 1877).

Actinomyces bovis was the first authentic actinomycete described as a causative agent of disease; it is natural, therefore, that it should have a number of synonyms. These are given here, without any guarantee that the list is complete.

Synonyms: *Discomyces bovis* Rivolta, 1878; *Bacterium actinocladothrix* Afanasiev, 1888; *Nocardia actinomyces* de Toni and Trevisan, 1889; *Actinomyces hominis* Bostroem, 1890; *Streptothrix actinomyces* Rossi-Doria, 1891; *Cladothrix bovis* Macé, 1891; *Oospora bovis* Sauvageau and Radais, 1892; *Actinomyces albidoflavus* Rossi-Doria, 1891; *Actinomyces sulphureus* Gasperini, 1894; *Nocardia bovis* R. Blanchard, 1895; *Streptothrix israeli* Kruse, 1896; *Cladothrix actinomyces* Macé, 1897; *Streptothrix actinomycotica* Foulerton, 1899; *Discomyces bovis* R. Blanchard, 1900; *Streptothrix spitzi* Lignières, 1903; *Sphaerotilus bovis* Engler, 1907; *Cohnistreptothrix israeli* Pinoy, 1911; *Actinomyces israeli* Vuillemin, 1931. See also Baldacci (1937).

Morphology: Grows in the form of sulfur-colored granules in the pus of cases of actinomycosis. The radiating hyphae are covered with extraneous material deposited by the host to form clubs. Organism is gram-positive, nonmotile, nonacid-fast. Colonies are dull white in color, only slightly adherent to the medium. No aerial hyphae. Mycelium undergoes fragmentation very rapidly into V- and Y-forms. Extensive branching is rare. Hyphae less than 1 μ in diameter (Fig. 3).

Semisolid media: Growth excellent, especially with paraffin seal. No soluble pigment produced.

Gelatin: Growth scant, flaky. No liquefaction.

Liquid media: Occasional turbidity with a light, flocculent growth.

Egg or serum media: No proteolytic action.

Milk: Turns acid; no coagulation and no peptonization. Sometimes there is no growth.

Sugar utilization: Acid from glucose, sucrose, and maltose; no acid from salicin or mannitol.

Temperature: Optimum 37°C. Does not grow at 22°C. Killed at 60°C.

Oxygen requirement: Anaerobic to microaerophilic. Grows readily in an atmosphere of CO_2. Bovine strains are more oxygen-tolerant on egg or serum media than strains of human origin.

Viability: Pure cultures do not live more than 10 to 14 days. On Dorset's egg medium, they may survive in an ice chest for 3 to 4 weeks.

Habitat: Originally found in lumpy jaw of

cattle. Usually found in and about mouths of animals.

Remarks: King and Meyer (1957) recently suggested that in order to implement proper identification of *A. bovis*, certain selected differential criteria, such as catalase test, litmus milk reactions, and the utilization of xylose, salicin, and raffinose, can be used. Slack and Moore (1960) suggested the use of fluorescent antibody formation for the further identification of this organism.

2. *Actinomyces baudetii* Brion, 1942 (Brion, G. de. Rev. de Méd. Vétér. **91:** 157, 1942; Brion, G. de, Goret, and Joubert. Proc. VI Congr. Intern. Patol. Comp., Madrid **1:** 48, 1952).

Morphology: Granules from histological preparations show tangled, radiating hyphae; ends of hyphae rounded and ovoid, forming a crown. Hyphae take basic stains. Mycelium composed of slender hyphae, 0.2 to 0.4 μ. Nonseptate. Ends swollen and rounded. Copious branching. In artificial media hyphae are frequently short, rarely exceeding 20 μ in length.

Agar colonies: Dull, whitish granules adhering slightly to the medium.

Liquid media: A sediment of white granules is produced.

Gelatin: No liquefaction.

Blood serum: In 4 to 5 days, surface covered with white granules which are the size of a pin head.

Serum media: No proteolytic action.

Brain extract: Growth favored in some media.

Indol: Production slight.

Sugar utilization: Acid from glucose, sucrose, and starch.

Oxygen demand: Anaerobic to microaerophilic.

Optimum temperature: 37°C.

Pathogenicity: Pathogenic when inoculated into dogs, rabbits, and guinea pigs (forms subcutaneous abscesses).

Source: Isolated from various types of lesions in cats and dogs.

3. *Actinomyces cellulitis* (Linhard, 1949) nov. comb. (Linhard, J. Ann. inst. Pasteur **76:** 478, 1949).

Synonym: *Actinobacterium cellulitis* Linhard.

Morphology: Polymorphic rods, showing primary, secondary, and sometimes tertiary branching. Length 5 to 7 μ, diameter 0.6 μ. Nonmotile. Gram-positive.

Agar media: Colonies lenticular. No gas.

Glucose broth cultures: No turbidity. Abundant growth, settling to bottom.

Gelatin: No liquefaction.

Milk: Unchanged.

Serum: Serophilic, but can be adapted to serum-free media.

Nitrate reduction: Positive.

Oxygen demand: Anaerobic and microaerophilic. Colonies produced at 4 to 5-mm depth in agar media.

Reduction: Does not reduce neutral red or safranin.

Carbon utilization: Positive utilization of glucose, fructose, maltose, galactose, and sucrose. Produces volatile acids (propionic and formic). Production of gas may suggest either a contaminated culture or the absence of an *Actinomyces*.

Pathogenicity: Nonpathogenic.

Habitat: Oral cavity of man.

4. *Actinomyces discofoliatus* (Grüter, 1932) Negroni (Negroni, P. Mycopathol. **1:** 81–87, 1938–1939).

Morphology: Deep colonies in semisolid glucose agar are whitish, lens-shaped, crossed or forming dihedral angles; margins of colonies regular; consistency of colonies slimy. Bacteria-like entities measuring 3 to 4 μ to 10 to 15 μ by 0.8 μ, occurring as isolated elements or V- or Y-shaped elements. Compact colonies in hanging-drop cultures. The filaments have a tendency to dichotomous

FIGURE 4. *A. israelii*, grown anaerobically in veal infusion agar, × 975 (Courtesy of Armed Forces Institute of Pathology).

branching, with prevailing development of one branch.

Glucose agar: Discoid, moist, and brilliant colonies; slightly elevated in the central part with nearly regular margins.

Gelatin: No liquefaction.

Glucose broth: Slimy sediment and sometimes a slight turbidity. The medium becomes clear at the end of 8 to 10 days.

Carbon sources: Acid but no gas from glucose, maltose, fructose, lactose, sucrose, and inulin; very little or no acid from mannitol.

Starch: Not attacked.

Sucrose: Not inverted.

Nitrate reduction: Negative.

H_2S: Formed.

Indol: Slight quantity produced.

Fats: Slightly attacked.

Olive oil: Not attacked.

Optimum temperature: 37°C.

Oxygen demand: Facultatively anaerobic.

Remarks: Vitality weak. Deep cultures in semisolid media die if held for longer than 8 to 10 days at 37°C, or for longer than 30 minutes at 60°C. Exposure for longer than a few minutes in dilute mineral acids kills the organism. The organism can be kept alive for 2 to 3 months if cultures are kept in an ice chest, in a dried state, or under vacuum.

Habitat: Lachrymal concretions and human actinomycotic lesions.

5. *Actinomyces hvidhanseni* (Hvid-Hansen, 1951) nov. comb. (Hvid-Hansen, N. Acta Pathol. Microbiol. Scand. **29:** 335–338, 1951).

Synonym: *Actinomyces israeli* Hvid-Hansen.

Morphology: Gram-positive, nonacid-fast, nonmotile. Polymorphic, bent, and often branched rods. Obligately anaerobic.

Meat liver agar: Colonies circular or irregular, often in the form of bodies bounded by four concave surfaces meeting in four acute vertices, of highly varying size and of a pale pink color. Surface colonies circular, convex, grayish-white or white; transparent S-colonies of a butyrous, viscous, but not mucous consistency.

Meat liver broth: Diffuse growth at first,

followed rapidly by a fairly voluminous pale pink, homogenous precipitate.

Thioglycollate medium: Growth either diffuse, netlike, or dispersed and granular.

Gelatin: Liquefied.

Milk: Coagulated in 24 to 48 hours and peptonized in 3 weeks.

Blood: All strains hemolyze human blood on solid media but do not form a soluble hemolysin.

Sugar utilization: Galactose, fructose, and glycerol vigorous; inulin, maltose, mannitol, saccharose, starch, dulcitol, and lactose somewhat less readily; xylose and arabinose not at all.

Reduction: Some strains form a little hydrogen sulfide. Sulfites and sulfates are not reduced. Nitrates reduced to nitrites and in some cases to ammonia. Safranin, phenosafranin, or neutral red not reduced. Most strains decolorize methylene blue in 4 to 24 hours; some do not.

Temperature: Optimum 37°C. Heating to 50–60°C for 15 minutes injurious.

Remarks: All strains catalase-positive. All produce ethyl alcohol, aldehyde, acetone, ammonia. A faint indol reaction is found in alkaline distillate. The presence of volatile acids, tartaric acid, and lactic acid has been demonstrated, but not succinic acid. Propionic acid and formic acid in ratios of from 3 to 1 up to 20 to 1 for the six strains examined.

Habitat: Ground water.

Remarks: Kalakoutskii (1960) found anaerobic actinomycetes in natural waters and in the air of apartments occupied by man, but not in the soil.

6. *Actinomyces israelii* (Kruse) Lachner-Sandoval, 1898 (Wolff, M. and Israel, J. Arch. pathol. Anat. **126:** 11, 1891).

Synonyms: *Streptothrix israeli* Kruse, 1896; *Discomyces israeli* Gedoelst, 1902; *Actinomyces bovis* Wright, 1905; *Discomyces bovis* Erumpt, 1906; *Actinobacterium israeli* Sampietro, 1908; *Cohnistreptothrix israeli* Pinoy, 1913; *Nocardia israeli* Castellani and Chalmers, 1913; *Brevistreptothrix israeli* Lignières, 1924; *Proactinomyces israeli* Jensen, 1931; *Corynebacterium israeli* Lentze, 1938; *Actinomyces israeli* var. *indo-sinensis* Reynes, 1947.

Morphology: Large, club-shaped forms are seen in morbid tissues. Substrate mycelium consists of rapidly septating and sporulating hyphae. The branches may extend into the medium in long filaments or may exhibit fragmentation and characteristic angular branching. Hyphae occasionally septate, but no definite spores are formed. Colonies exhibit a considerable degree of polymorphism, but no stable variants have been established. Colonies are tougher in texture than those of *A. bovis*. Old colonies warted in appearance (Fig. 4).

Gelatin: Growth scant, flaky. No liquefaction.

Liquid media: Growth in form of white compact colonies or granular sediment. Medium shows no turbidity, usually remaining clear. No gas and no odor.

Pigments: No soluble or insoluble pigments.

Egg media: No proteolytic action.

Milk: Becomes acid, but usually does not clot. No peptonization. Frequently no growth.

Starch: Slight hydrolysis.

Oxygen requirement: Anaerobic.

Nitrate reduction: Generally negative.

Sugar utilization: Greater ability to utilize sugars than *A. bovis*. Acid but no gas from glucose, galactose, lactose, fructose, maltose, raffinose, sucrose; no acid from inulin.

Hemolysis: Slight to marked.

Serological reactions: Lack of serological affinity with *A. bovis*.

Temperature: Optimum 37°C. Destroyed at 55–60°C in 30 minutes.

Habitat: Dental caries, tonsils, and natural cavities of man and animals. Chief etiological agent of human actinomycosis, de-

FIGURE 5. *A. israelii* (Reproduced from: Prévot, A. R. 6th Intern. Congr. Microbiol., Symp. Actinomycetales, Rome, 1953, p. 45).

scribed first by Wolff and Israel (1891) and later by Wright (1905).

Remarks: Vitality weak. Cultures no longer viable after 8 to 10 days. Erikson and Porteous (1953) succeeded in obtaining good growth by continued subculture in a medium containing 99 parts of 1 per cent casein hydrolyzate and 1 part of heart broth and 0.5 per cent glucose. Antigenic structure of organism has been recently studied by Kwapinski (1960).

According to Grootten (1934), the organism is highly polymorphic. Rods varying in length are formed in young culture. They are straight or slightly curved, with round or oval extremities. Occasionally, long or even filamentous forms are found. Some of the filaments end in small spherical or pear-shaped swellings. It does not form spores. In agar tubes, it does not grow in the upper 5- to 10-mm zone; below that zone, it forms a layer of 2 to 4 mm with numerous small colonies; in the deeper layers, the colonies are fewer, but may attain diameters of 2 to 3 mm. No gas and no odor are produced. Liquid media remain clear. The organism is nonproteolytic; milk is not coagulated. Blood is rapidly hemolyzed. It does not grow on potato plugs, except poorly when glycerinated. It slowly attacks glucose, lactose, maltose, sucrose, and mannitol. It does not grow in glucose-gelatin medium. Animal infection is obtained by introducing a culture into the peritoneum of rabbits.

Negroni (1954) described *A. israelii* in further detail. Deep colonies in semisolid glucose agar are globous, 1 to 2 mm in diameter, whitish, opaque, and with an irregular surface. Colonies are of a cheesy consistency and cannot be homogeneously suspended in water. On glucose or glycerol agar slants, the colonies are elevated, mammilated, and whitish, with moist and brilliant surface and irregular margins. Submerged mycelium is well developed. The colonies have a cheesy consistency and can easily be removed from the medium with a platinum loop (Fig. 5).

According to Erikson and Porteous (1955), the conversion of a "rough" typical strain of *A. israelii* to a "smooth" soft form more tolerant of oxygen is a result of the physical trapping within the mycelium of a few alien facultative anaerobes, usually staphylococci.

7. *Actinomyces naeslundii* Thompson and Lovestedt, 1951 (Thompson, L. and Lovestedt, S. A. Proc. Staff Meet. Mayo Clinic **26:** 169, 1951).

Morphology: Organism forms small, whitish, firm colonies. Mycelial branching, but no segmentation. Not acid-fast.

Artificial media: Good growth.

Hormone agar: Rough and smooth colonies, 1 to 2 mm in diameter, after 4 days. Surface of colonies varies from smooth to nodular to wrinkled. Consistency varies from butyraceous to tough and adherent. Colonies are opaque, with color varying from white to cream.

Glucose brain broth: Growth rapid and abundant. Acid produced.

Gelatin: Growth slow. No liquefaction.

Starch: Not hydrolyzed.

Milk: Growth scant or absent.

Aerobiosis: Grows both under aerobic and anaerobic conditions, somewhat better aerobically.

Temperature: Optimum at 37°C; some growth at 32°C.

Pathogenicity: Nonpathogenic.

Habitat: Human mouth. Considered to be a saprophyte found in the mouth and frequently confused with *A. israelii*.

8. *Actinomyces odontolyticus* Batty, 1958 (Batty, I. J. Pathol. Bacteriol. **75:** 455–459, 1958).

Morphology: At first, the organism appears in the form of short rods subdivided by one or two transverse septa. Later, these rods gradually elongate until a septate submycelium is produced. At the end of these filaments, globular "initial cells" are produced which germinate to produce a nonseptate secondary submycelium, which soon commences to break up. Finally, in a week to 10 days, small spores commence to form singly upon short side branches. The size of the mature spores varies greatly in different strains.

Appearance of colonies: Colonies are usually few in number with an initial appearance similar to those of α-hemolytic streptococci of comparable age. Later, they develop a dark red hemin-like pigment, easily distinguishable. At and after this stage the colonies are exceedingly difficult to subculture. After prolonged artificial culture the organisms can be subcultured at any stage. Attempts to isolate the organism upon horse serum agar or nutrient agar are usually unsuccessful, but after several subcultures a profuse growth of small convex nonpigmented colonies is obtained on both these media. All strains grow equally well under aerobic and anaerobic conditions at all stages in their life cycle; in agar stab cultures a filiform growth is obtained throughout the line of inoculum. Growth in peptone broth is sparse, but in this medium enriched with yeast extract, a characteristic glutinous ropy sediment is produced which disperses to give an even turbidity (Fig. 6).

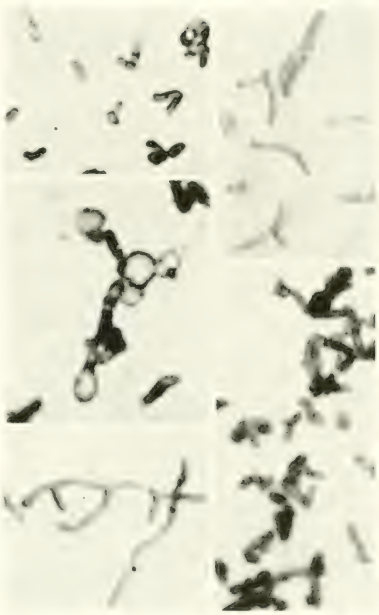

FIGURE 6. *A. odontolyticus*, various stages of culture development (Reproduced from: Batty, I. J. Pathol. Bacteriol. **75:** 455–459, 1958).

No strain produces catalase, oxidase, indole, hydrogen sulfide or acetylmethylcarbinol; all are methyl red-negative and all fail to ferment fructose, maltose, trehalose, starch, inulin, dextrin, glycogen, xylose, rhamnose, glycerol, dulcitol, and salicin. A few strains ferment sucrose, galactose, arabinose, or mannitol, with the production of acid but no gas. About half the strains produce ammonia from peptone, acidify and coagulate litmus milk, and are tolerant of a concentration of 1 in 4000 potassium tellurite. Some strains hydrolyze urea. None liquefy gelatin, Loeffler's medium, or coagulated egg medium. All reduce nitrate to nitrite within 18 hours.

Habitat: Human saliva in deep dental caries.

Remarks: This organism resembles *A. bovis* in its life cycle.

Incompletely Described Forms of *Actinomyces*

In addition to the above well described and readily recognizable forms belonging to the genus *Actinomyces*, numerous other anaerobic organisms have been listed in the literature. Some of these organisms are no doubt strains of the well described forms and their names would be in synonymy. Others may represent distinct species.

It is of particular interest to cite, in this connection, the ideas of Prévot (1957), who considered *Actinomyces bovis* as an aerobic organism and, therefore, the genus *Actinomyces* as an aerobic group. He suggested that *Actinomyces israelii* represent the anaerobic group, and the generic name *Actinobacterium* Haas, 1906 (Syn. *Cohnistreptothrix* Pinoy, 1913) be given priority for designating the anaerobic forms. Prévot (1957) divided the genus *Actinobacterium* into six species: (1) *A. israelii*, (2) *A. meyeri*, (3) *A. abscessus*, (4) *A. liquefaciens*, (5) *A. cellulitis*, and (6) *A. propionici*.

In addition to the above, numerous other anaerobic forms have been described, such as *A. canis* Levy, 1899; *A. interproximalis* Fennel, 1918; and others. Some additional names will be found in Chapter 13.

Chapter 3

The Genus *Nocardia*

Characterization of Genus

The genus *Nocardia* represents a group of aerobic actinomycetes which includes both pathogens and saprophytes. The relationship of this genus to, and possible overlapping with, the genus *Mycobacterium*, on the one hand, and the genus *Streptomyces*, on the other, have already been discussed (Volume I). Numerous cultures of nocardiae have been isolated from human and animal infections, and claimed to be the causative agents of the particular disease. The fact, however, that a culture of an organism has been isolated from a lesion of a man or an animal is no proof that it is primarily responsible for the particular disease; it may actually be a secondary invader or a member of a mixed infection. Some species of *Nocardia* are definitely associated, however, with certain diseases, or have at least been isolated from infected tissues. This gave origin to the term "nocardiosis," descriptive of these disease conditions.

The colonies produced by nocardiae are either smooth, or rough and much folded; they are either of a soft or dough-like consistency, or compact and leathery, especially in early stages of growth. Many species of *Nocardia* do not form any aerial mycelium; some give rise to a limited aerial mycelium which may structurally be similar to that of the substrate mycelium; still others may produce aerial hyphae and spores which may be indistinguishable from those of *Streptomyces* and are thus responsible for various cases of overlapping between these two genera.

Nocardias multiply by concentration and segmentation of the protoplasm within a filamentous cell, followed by dissolution of the cell membrane. The fragmented portions of the mycelium usually develop into fresh mycelium under favorable conditions, either by germ tubes or by lateral budding. Streptomycetes produce true spores or conidia, the vegetative mycelium not segmenting spontaneously into bacillary or coccoid forms, but remaining nonseptate and coherent even in old cultures, thus producing the characteristic tough textured, leathery growth. In nocardiae, the aerial mycelium represents an extension upward of the vegetative mycelium; it does not exhibit any differentiated protoplasm and is sterile and abortive. When a streptomycete has lost the capacity of producing aerial mycelium, a form analogous to that of a nocardia may result, except for the structure of the mycelium and the capacity of the degenerated streptomycete to regain the lost capacity. It is occasionally, therefore, a matter of personal preference whether to place a freshly isolated culture in one genus or another. Some nocardiae are acid-fast or partially acid-fast, and others are not.

The mode of branching of the substrate mycelium (see Volume I, Chapter 5), the biochemical properties (proteolytic and serological activities), and chemical nature of the cell walls of nocardiae appear to distinguish them from the streptomycetes. Hoare and

Work (1957) have shown that these genera can be differentiated by the configuration of the diaminopimelic acid present in whole cell hydrolysates; streptomycete cell walls contain the L-isomer, whereas nocardiae cell walls contain the DL-isomer. Cummins and Harris (1958) reported that the presence or absence of arabinose in the hydrolysates of the intact organisms can also be used to identify them; nocardiae cell walls contain arabinose, whereas streptomycete cell walls do not. The sensitivity of most *Streptomyces* species, but not of nocardiae, to the action of lysozyme on their cell wall preparations, studied by Sohler, Romano, and Nickerson (Volume I, p. 159), provides further criteria for distinguishing between members of these two genera. Studies of infrared absorption as a taxonomic criterion (Riddle *et al.*, 1956) has also been suggested.*

In view of the overlapping between certain forms placed for convenience in either one genus or the other, the separation of atypical strains of *Nocardia* or *Streptomyces* by morphology or fermentation tests alone may be difficult, as pointed out by Gordon and Mihm (1957).

The genus *Nocardia* has been described in the last edition of Bergey's Manual as follows:

"Slender filaments or rods, frequently swollen and occasionally branched, forming a mycelium which, after reaching a certain size, assumes the appearance of bacterium-like growths. Shorter rods and coccoid forms are found in older cultures. Conidia not formed. Stain readily, occasionally showing a slight degree of acid-fastness. Nonmotile.† No endospores. Aerobic. Gram-positive. The colonies are similar in gross appearance to those of the genus *Mycobacterium*. Paraffin, phenol and *m*-cresol are frequently utilized as a source of energy.

"In their early stages of growth on culture media (liquid or solid), the structure of nocardias is similar to that of actinomycetes in that they form a typical mycelium; hyphae branch abundantly, the branching being true. The diameters of the hyphae vary between 0.5 and 1 μ, usually 0.7 to 0.8 μ, according to the species. The mycelium is not septate. However, the further development of nocardias differs sharply from that of actinomycetes; the filaments soon form transverse walls and the whole mycelium breaks up into regularly cylindrical short cells, then into coccoid cells. On fresh culture media, the coccoid cells germinate into mycelia. The whole cycle in the development of nocardias continues for 2 to 7 days. Most frequently the coccoid cells are formed on the third to fifth day, but in certain species they can be found on the second day.

"The multiplication of nocardias proceeds by fission and budding; occasionally they form special spores. Budding occurs often. The buds are formed on the lateral surface of the cells; when they have reached a certain size, they fall off and develop into rod-shaped cells or filaments. The spores are formed by the breaking up of the cell plasm into separate portions usually forming 3 to 5 spores; every portion becomes rounded, covered with a membrane and is transformed into a spore; the membrane of the mother cell dissolves and disappears. The spores germinate in the same way as those of actinomyces. They form germ tubes which develop into a mycelium (Fig. 7).

"The colonies of nocardias often have a paste-like or mealy consistency and can easily be taken up with a platinum loop; they spread on glass and occasionally render the broth turbid. The surface colonies are

* Personal communication from Dr. N. M. McClung.

† The existence of motility among the nocardias was considered by Jensen (1953) as indisputable, and this really is not surprising in view of the numerous observations on motility in the closely related coryneform bacteria. The species in the order *Actinomycetales* cannot any longer be regarded as constantly nonmotile (Fig. 14).

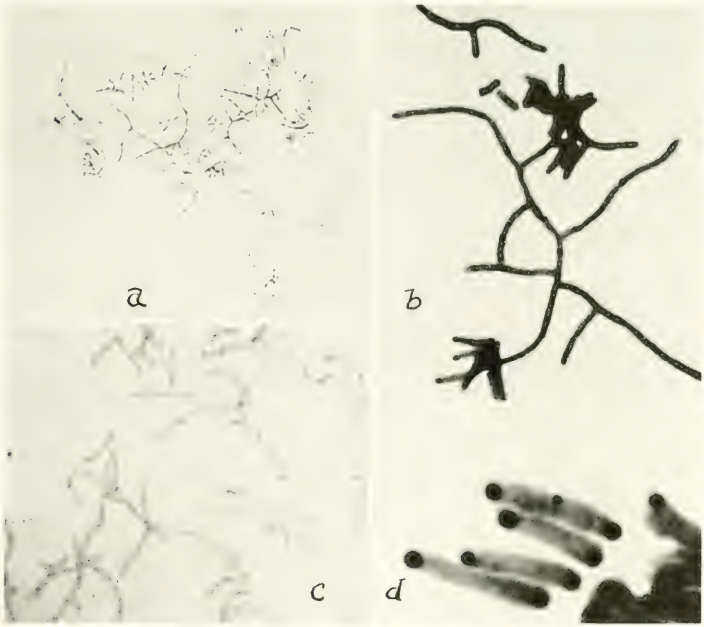

FIGURE 7. *N. opaca:* (a) grown for 4 days on *n*-dodecane and mineral salts; gram stain, × 960; (b) same grown 3 days; × 3700; (c) same as (a) but using fat stain, × 1920; (d) two-day growth, × 12,500 (Reproduced from: Webley, D. M. J. Gen. Microbiol. 11: 425, 1954).

smooth, folded or wrinkled. Typical nocardias never form an aerial mycelium, but there are cultures whose colonies are covered with a thin coating of short aerial hyphae which break up into cylindrical oidiospores.* Many species of nocardias form pigments; their colonies are of a blue, violet, red, yellow or green color; more often the cultures are colorless. The color of the culture serves as a stable character. The type species is *Nocardia farcinica* Trevisan."

Classification of *Nocardia* Species

De Toni and Trevisan (1889) described five species of *Nocardia*: *N. farcinica*, *N. actinomyces*, *N. foersteri*, *N. arborescens*, and *N. ferruginea*.

Jensen (1932a) found that a number of organisms previously described as species of *Mycobacterium* actually belong, on account of definite mycelial growth in the initial stages of their life cycles, to the genus *Nocardia.** *Mycobacterium agreste* Gray and Thornton and *B. mycoides corallinus* Hefferan were found to be similar to one another and were regarded as one species, *N. corallina*. The same was true of *M. salmonicolor* den Dooren de Jong, which was designated as *N. salmonicolor*. *Mycobacterium opacum* den Dooren de Jong and *M. crystallophagum* Gray and Thornton proved to be identical and were named *N. opaca*. *Mycobacterium erythropolis*, a closely related form, was des-

* See the work of Gordon and Mihm (1958).

* Generic name *Proactinomuces* used.

FIGURE 8. *N. paraffinae,* showing a section of a colony covered with mature aerial mycelium (Reproduced from: Hirsch, P. and Engel, H. Ber. Deut. Botan. Ges. **69:** 454, 1956).

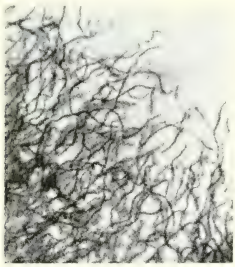

FIGURE 9. *Nocardia,* strain 70, showing the development of the aerial mycelium on mineral agar (Reproduced from: Hirsch, P. and Engel, H. Ber. Deut. Botan. Ges. **69:** 454, 1956).

ignated as *N. erythropolis. Microbacterium mesentericum* Orla-Jensen was renamed *N. mesentericus.*

Jensen divided the genus *Nocardia* into two distinct groups:

I. Nonproteolytic organisms with strongly refractive cells showing a partial acid-fastness in milk and sometimes in other media; capable of decomposing paraffin. Some species of this group form a transition to the genus *Mycobacterium.*

II. Mostly proteolytic forms with weakly refractive, nonacid-fast cells. This group forms a close transition to the forms now included in the genus *Streptomyces.*

A further separation of the genus was based upon the structure of the aerial mycelium.

A. Unstable mycelium (α-form), with short mycelium (if formed at all), bacterial (diffuse) growth in liquid media, bacteria-like colony.

B. Stable mycelium (β-form), producing long hyphae, colony growth in liquid media, and *Streptomyces*-like type of colony.

Umbreit (1939) modified the system of Jensen as follows:

I. Partially acid-fast, nonproteolytic, nondiastatic; constantly utilize paraffin.
   1. α-Mycelium type: *N. opaca,* N. erythropolis.*
   2. β-Mycelium type:
      a. Red-colored: *N. polychromogena N. asteroides.*
      b. Yellow-colored: *N. paraffinae.*

II. Nonacid-fast forms, constantly diastatic
   1. α-Mycelium type:
      a. Nonproteolytic: *N. mesenterica.*
      b. Proteolytic: *N. actinomorpha.*
   2. β-Mycelium type:
      a. Yellow-colored: *N. flavescens.*
      b. Red to orange: *N. maculata.*

Krassilnikov (1938) divided the genus *Nocardia** into two groups:

I. Well developed aerial mycelium, with substrate mycelium seldom producing cross walls. The hyphae break up into long, thread-like rods. Branches of the aerial mycelium produce segmentation spores and oidiospores; the latter are cylindrical with sharp ends. No spirals or fruiting branches. This is the same as group β of Jensen.

II. Typical nocardial forms. Mycelium develops only at early stages of growth, then breaks up into rod-shaped and coccoid bodies. Smooth and rough colonies, dough-like consistency, similar to bacterial colonies. Aerial mycelium not formed or only around colonies.

* Generic name *Proactinomyces* used.

TABLE 1

Summary of growth characteristics of 18 strains of Nocardia (McClung, 1949)

Organism	Germination	Primary branching	Secondary branching	Fragmentation			
				Age	Type 1	Type 2	Type 3
	hr	*hr*	*hr*	*hr*	%	%	%
Group I							
W-F	8	16	0	13	70	30	0
N. aquosus	6	12	0	12	60	30	10
KLJ	9	15	0	13	50	50	0
B-B	10	36	0	14	50	50	0
N. erythropolis	11	14	0	14	69	40	0
N. polychromogenes	11	13	0	14	40	30	30
Group II							
13-20	11	12	5	19	6	28	66
43-8	10	12	7	18	5	40	50
N. ruber	10	14	4	20	5	30	65
N. polychromogenes	14	30	20	120	Unknown		
N. asteroides	10	28	96	96	Unknown		
Group III							
21-3	10	16	4		0	0	0
13-10	9	11	6		0	0	0
7-7	10	12	8		0	0	0
18-2	11	15	6		0	0	0
13-15	10	15	5		0	0	0
13-3	10	13	9		0	0	0
20-6	8	13	7		0	0	0

McClung (1949) divided the genus *Nocardia* into three groups:

I. Scant mycelial development, sparse branching. Colonial texture soft, pasty, and sometimes mucoid; pigment intracellular and insoluble.

II. Extensive mycelial development, straight branches which do not overlap. Colonial texture soft and pasty; pigment intracellular and insoluble.

III. Extensive mycelial development, no fragmentation of hyphae, contorted and profusely produced branches which overlap. Colonial texture waxy or cartilaginous; generally both intracellular and soluble pigments are produced (Figs. 8, 9).

The pattern in *Nocardia* fragmentation can be separated into three types. In Type 1, an acute angle is formed in a hypha preceding division, which occurs at the apex of the bend; following division the new hyphal tips grow out parallel to each other. In Type 2, division occurs in a straight or slightly curved portion of a hypha; following division, the newly formed ends bend slightly and grow past each other. In Type 3, division occurs in the parent hypha close to or at the juncture of a branch; a new hypha may grow from the place of division at the base of the branch; the newly formed hyphal tip bends and continues to grow. Type 1 fragmentation is characteristic of Group I, and Type 3 of Group II. Type 2 occurs in both groups, and Group III lacks fragmentation (Fig. 10).

A summary of the growth characteristics of various strains of *Nocardia* belonging to these three groups is presented in Table 1.

In an attempt to find a group of dependable properties for the separation of the genera *Nocardia*, *Streptomyces*, and *Myco-*

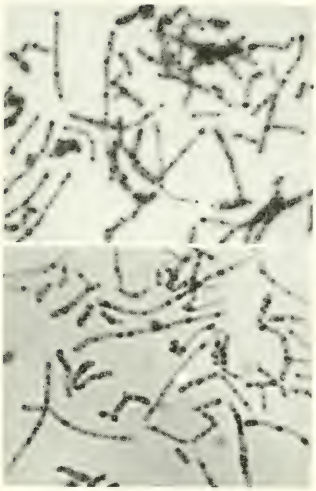

FIGURE 10. *N. rubra*: (above) 24 hr, glycerol nutrient agar, methylene blue; (below) same, stained with Sudan black B, × 1600 (Reproduced from: McClung, N. M. Lloydia **12:** 165, 1949).

bacterium, Gordon and Smith (1955) examined 152 cultures labelled *Streptomyces* and 99 cultures labelled *Nocardia*; those strains of the latter that formed soft, fragmenting, vegetative mycelium were excluded. Of the cultures designated as *Streptomyces*, 83 per cent produced an aerial mycelium typical of this genus; 13 per cent failed to produce aerial mycelium, although a few formed rudimentary aerial hyphae; inability to form spores was considered as a lost property, since the physiological reactions of the strains were the same as in the previous group of sporulating cultures; only five strains, or 4 per cent of the cultures possessed nocardial properties. Of the cultures designated as *Nocardia*, 68 produced aerial hyphae, varying from rudimentary to luxuriant, some even forming chains of spores. According to their physiological properties, 24 of these cultures should have been designated as *Streptomyces*. A few of the strains could be considered as intermediate between the two genera.

Gordon and Mihm (1957) further reported the results of an examination of 219 cultures labelled *Streptomyces*, 214 *Nocardia*, and 243

TABLE 2

Certain physiological and biochemical characteristics of various strains of two species of Nocardia (Gordon and Mihm, 1959)

Property	*N. asteroides* (98 strains), positive strains	*N. brasiliensis* (50 strains), positive strains
	%	%
Decomposition of:		
Casein	0	98
Gelatin	36	100
Tyrosine	0	100
Xanthine	0	0
Hydrolysis of starch	58	56
Acid from:		
Adonitol	0	0
Arabinose	0	0
Erythritol	6	0
Galactose	24	92
Glucose	100	96
Glycerol	98	98
Inositol	2	100
Lactose	0	0
Maltose	6	0
Mannitol	0	94
Mannose	19	68
α-Methyl-D-glucoside	0	0
Raffinose	0	0
Rhamnose	33	0
Sorbitol	0	0
Xylose	0	0
Nitrite from nitrate	85	92
Growth at:		
50°C	24	0
40°C	90	56
35°C	100	100
28°C	100	100
10°C	12	30
Utilization of:		
Acetate	100	100
Citrate	33	98
Malate	100	100
Propionate	100	100
Pyruvate	100	100
Succinate	100	98
Benzoate	0	0

TABLE 3

Comparative properties of certain acid-fast nocardias (Suter, 1951)

	Aerobic	Pigment production	Growth on potato	Aerial mycelium	Growth at room temperature
N. fastidiosa	−	−	−	−	−
N. leishmanii	+	+		−	
N. caprae	+	+	+	−	
N. pretoriana	+	−	+	−	
N. pulmonalis	+		+	+	
N. paraffinae	+	−	+	+	
N. transvalensis	+	+	+	+	
N. polychromogenes	+	+		−	+
N. minima	+	+	+	−	+
N. coeliaca	+	+		−	+
N. rubropertincta	−	+	+	−	
N. asteroides	+	−	+	−	−
N. salmonicolor	+	+	+	−	
N. rubra	+	+		−	
N. farcinica	−	+	+	−	−

Mycobacterium. In the case of the *Streptomyces*-designated cultures, 83 per cent produced sporulating aerial hyphae, 9 per cent nonsporulating aerial hyphae, and 8 per cent formed no aerial hyphae. The *Nocardia*-designated cultures gave, with regard to production of aerial hyphae, 24, 47, and 10 per cent, respectively. Among the 214 *Nocardia*-designated cultures, 79 were recognized as representing *N. asteroides* (Eppinger) Blanchard. They all produced acid from glucose and glycerol, and utilized acetate, malate, propionate, pyruvate, and succinate. They all grew well at 28 and 35°C, and 88 per cent grew at 40°C. Eighty-six per cent reduced nitrate to nitrite, 54 per cent hydrolyzed starch, and 34 per cent decomposed gelatin. A large number of cultures designated as *Nocardia* (*N. corallina*, *N. erythropolis*, *N. globerula*, *N. lutea*, *N. opaca*, *N. rhodnii*, *N. rubra*) were tentatively assigned by Gordon and Mihm to the mycobacteria under *M. rhodochrous* (Overbeck) nov. comb. (Table 2).

Of five species of aerobic actinomycetes associated with various mycetomas, Mariat (1957) recognized only *N. asteroides* and *N. brasiliensis* as nocardiae; *Streptomyces madurae*, *S. pelletieri*, and *S. somaliensis* were considered as streptomycetes, although Mariat was not quite certain of their exact systematic position.

Bojalil and Cerbon (1959) divided the genus *Nocardia* into two different metabolic groups: (1) Produces round colonies, adhering to wall and bottom of tube; utilizes gelatin as the only source of N and C, breaking it down into amino acids and giving an alkaline reaction. *N. brasiliensis* belongs to this group. (2) Produces flaky growth easily dispersed through medium; poor growth on gelatin. *N. asteroides* belongs to this group.

A detailed examination of the variability of different strains of two species of *Nocardia* with regard to their ability to utilize different carbon and nitrogen sources, as well as in certain other physiological and biochemical properties, is reported in Table 2. Some comparative properties of several nocardiae are given in Table 3.

Spalla (1958, 1959) criticized the various descriptions of *Nocardia* species on the basis of an insufficient number of characters. He suggested that the following properties be used for characterization and classification of nocardiae:

TABLE 4

Cultural and biochemical properties of certain species of Nocardia (Spalla, 1959)

Organism	Acid production from:															Other properties						Growth* on		
	d-Galactose	*d*-Ribose	Rhamnose	*d*-Mannose	*l*-Arabinose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Inulin	Glycerol	*d*-Mannitol	Adonitol	*d*-Sorbitol	Reduction of nitrate	Hydrolysis of starch	Hydrolysis of gelatin	Coagulation of milk	Acid resistance	Aerial mycelium	N-Z amine medium	Glycerol agar	Glucose-as paragine agar
N. rugosa	+	+	+	−	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	−	−	C	C	C
Strain 279	+	+	+	−	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	−	−	C	C	C
Strain 959	+	+	+	−	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	−	−	C	C	C
Strain 25	+	+	+	+	+	−	−	−	−	−	−	+	+	+	−	−	−	+	+	−	−	C	C	C
N. asteroides	−	−	−	+	−	+	−	−	+	−	−	+	+	−	+	+	−	+	−	+	−	R	R-S	R-L
N. blackwellii	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	Y-A	C	Y R
N. rubra	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	Y-A	O	Y-O
N. gardneri†	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	+	+	+	+	−	+	C	C	C

* Y = yellow; O = orange; C = colorless; R = rose; L = lilac; A = apricot; S = salmon.

† Now recognized as a *Streptomyces*.

TABLE 5

Serological relations of major pathogenic actinomycetes (González Ochoa and Vasquez Hoyos, 1953)

Antigens	Precipitin formation					
	N. brasiliensis No. 447	*N. asteroides* No. 19	*S. madurae* No. 412	*S. pelletieri* No. 1185	*S. somaliensis* No. 1065	*S. paraguayensis* No. 285
A. bovis No. 527	+	+	−	−	−	−
N. brasiliensis No. 447	+	+	−	−	−	−
N. brasiliensis No. 468	+	+	−	−	−	−
N. mexicana No. 414	+	+	−	−	−	−
N. asteroides No. 18	+	+	−	−	−	−
N. asteroides No. 19	+	+	−	−	−	−
N. asteroides No. 20	+	+	−	−	−	−
N. asteroides No. 652	+	+	−	−	−	−
N. asteroides No. 694	+	+	−	−	−	−
N. gypsoides No. 9911	+	+	−	−	−	−
N. carnea No. 616	+	+	−	−	−	−
N. leishmanii No. 1030	+	+	−	−	−	
S. madurae No. 412	−	−	+	+	−	−
S. pelletieri No. 1185	−	−	+	+	−	−
S. africana No. 615	−	−	+	+	−	−
S. somaliensis No. 1065	−	−	−	−	+	−
S. somaliensis No. 1064	−	−	−	−	+	−
S. somaliensis No. 1066	−	−	−	−	+	−
S. paraguayensis No. 285	−	−	−	−	−	+
S. albus No. 693	−	−	−	−	−	+
S. griseus No. 975	−	−	−	−	−	+
S. lavendulae No. 9963	−	−	−	−	−	+

1. Color of growth on several synthetic and at least one organic medium.
2. Acid production from various carbon sources.
3. Heat resistance.
4. Size of terminal fragments.
5. Staining properties, notably acid-fastness.
6. Diastase formation.
7. Gelatin liquefaction.
8. Reduction of nitrate to nitrite.
9. Coagulation and peptonization of milk.
10. Formation of aerial mycelium.

By utilizing these properties and considering the high degree of similarity, Spalla was able to conclude that three mutants of *N. rugosa* belonged to the same species as the parent, and that *N. rugosa*, *N. asteroides*, *N. rubra*, and *N. blackwellii* represent distinct species, with a low degree of similarity.

A summary of some of the properties of *N. rugosa* and three of its mutants, as well as of certain other *Nocardia* species, is given in Table 4. The marked difference between the culture designated as *N. gardneri* and the other nocardiae may be noted, particularly in utilization of carbon sources, starch hydrolysis, and formation of aerial mycelium. These differences account for the fact that *N. gardneri* is now recognized as a *Streptomyces* and not as a *Nocardia*.

Various serological reactions of different species of *Streptomyces* and *Nocardia* are given in Table 5. Further information on the relation of *Mycobacterium* to *Nocardia* is found in the work of Haag (1927) and Gordon (1937).

Of the various treatments of the genus *Nocardia*, the following three systems of classification of species are presented here:

1. Classification of *Nocardia** Species, According to Jensen (1932a)

A. Partially acid-fast organisms with strongly refractive cells; nonproteolytic, generally nondiastatic; capable of utilizing paraffin.
  I. Initial mycelium limited, rapidly dividing into rods and cocci.
    1. Slowly growing organism; cells 0.5 to 0.7 μ in diameter.
      Nocardia minima
    2. Rapidly growing organisms; cells 1.0 to 1.2 μ in diameter.
      a. Cystites not produced; rapid formation of cocci.
        Nocardia corallina
      b. Cystites produced; less rapid formation of cocci.
        Nocardia salmonicolor
  II. Initial mycelium well developed, richly branching, dividing into rods and generally into cocci.
    1. Substrate growth soft, without macroscopically visible aerial mycelium.
      a. Substrate growth red; may produce variants with undivided substrate mycelium and visible white aerial mycelium, or yellow and white variants.
        Nocardia polychromogenes
      b. Substrate growth white to pale pink.
        a¹. Growth in nutrient agar opaque, cream-colored; cocci in broth culture.
          Nocardia opaca
        b¹. Growth on sugar-free nutrient agar watery; no cocci in broth culture.
          Nocardia erythropolis
    2. Substrate growth hard, yellow, with white aerial mycelium; sporophores divide into chains of acid-fast cocci.
      Nocardia paraffinae

B. Nonacid-fast organisms with weakly refractive cells; no distinct formation of cocci. Diastatic.
  I. Nonproteolytic; no aerial mycelium; marked production of cystites.
    Nocardia mesenterica
  II. Proteolytic organisms.
    1. Growth on nutrient agar with rapid formation of unbranched diphtheroid-like rods; no typical cystites; broth turbid.
      Nocardia actinomorpha
    2. Growth with extensive mycelium on nutrient agar; simple unbranched rods not formed; cystites present; broth clear.
      Nocardia flavescens

* Designated by Jensen as *Proactinomyces*.

2. Classification of *Nocardia*,* According to Krassilnikov

A. Cultures colorless, some excreting a brown substance into the medium.
  I. Aerial mycelium and spore-bearing hyphae produced in culture media.
    1. Substrate and aerial mycelium occasionally forming septae; the hyphae break up into long rods, 15 to 30 μ; spherical bodies not formed.
      a. Saprophytes, found on dead substrates.
        Nocardia actinoides
      b. Parasites, living in bodies of man and animals.
        Nocardia gedanensis
    2. Mycelium producing frequent septae; hyphae break up into short rods and cocci.
      a. Saprophytes.
        a¹. Grow on protein media.
          Nocardia actinomorpha
        b¹. Grow on paraffin.
          Nocardia paraffinae
      b. Parasites.
        Nocardia bovis
  II. Cultures produce aerial sporophores on the surface of the colonies, but no aerial mycelium; sporophores are short, straight, covering the surface of the colonies with a thin, pale layer.
    1. Cultures grow in organic media.
      Nocardia albicans
    2. Cultures grow in inorganic media.
      Nocardia oligocarbophilus
  III. Cultures not forming any sporophores or any aerial mycelium; colonies smooth or lichenoid.
    1. Saprophytes found on dead substrate.
      Nocardia albus
    2. Parasites or symbionts living within plants, animals, or man.
      a. Organisms living in symbiosis with plants, forming nodules on their roots.
        Nocardia alni
        Nocardia myricae
        Nocardia elaeagnii

* Generic name *Proactinomyces* used.

b. Organisms living in the bodies of man and animals.
a¹. Anaerobes, living in absence of oxygen.
Nocardia anaerobicus
b¹. Aerobes, or microaerophilic forms.
a². Strict aerobes.
Nocardia lignieresi
b². Facultative aerobes.
a³. Cells nonacid-fast.
*Nocardia israeli**
b³. Cells acid-fast.
Nocardia muris

B. Cultures pigmented.
I. Cultures pigmented violet or blue, the pigments diffusing into the substrate.
1. Well developed substrate mycelium produced, hyphae forming occasional septae and breaking up into long rods, 20 to 30 μ; colonies form a faint aerial mycelium with straight sporophores; spores cylindrical.
Nocardia gabritschewski
2. No aerial mycelium produced, hyphae or substrate mycelium forming frequent septae and breaking up into short rods and cocci.
Nocardia cyaneus
II. Red or orange pigment produced.
1. Mycelium forming occasional septae and breaking up into long rods; some give rise to a faint aerial mycelium and short straight sporophores.
a. Saprophytes.
a¹. Cultures not forming any soluble pigment in medium.
Nocardia fructiferi
b¹. Cultures producing a brown substance.
Nocardia polychromogenes
b. Parasites living in bodies of man and animals.
Nocardia freeri
2. Hyphae forming frequent septae and breaking up into short rods and cocci; no aerial mycelium produced.
a. Saprophytes living on dead substrates.
Nocardia ruber
b. Parasites living in bodies of man and animals.

* See Chapter 2 for description of *Actinomyces israelii*.

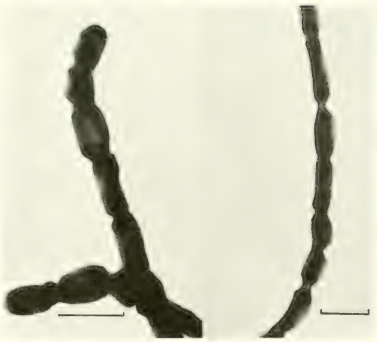

FIGURE 11. *N. asteroides*, strain 730, grown on soil extract agar (Reproduced from: Gordon, R. E. and Mihm, J. M. J. Bacteriol. **75**: 240, 1958).

a¹. Cells acid-fast.
Nocardia asteroides
b¹. Cells nonacid-fast.
Nocardia variabilis
III. Cultures citron-yellow or bright yellow.
1. Faint aerial mycelium with straight sporophores and cylindrical spores produced.
a. Saprophytes.
Nocardia flavescens
b. Parasites.
*Nocardia somaliensis**
2. No aerial mycelium produced.
a. Saprophytes.
a¹. Cultures yellow or bright yellow.
Nocardia flavus
b¹. Cultures citron-yellow.
Nocardia citreus
b. Parasites.
a¹. Cells acid-fast.
Nocardia farcinica
b¹. Cells nonacid-fast.
Nocardia putoria
IV. Cultures pigmented green.
1. Saprophytes.
Nocardia viridis
2. Parasites.
Nocardia pyogenes
V. Cultures black.
1. Saprophytes.
Nocardia nigra
2. Parasites.
Nocardia sendaiensis

* Now recognized as a *Streptomyces*.

3. Classification of *Nocardia*, According to the system of Waksman and Henrici*

A. Partially acid-fast organisms with strongly refractive cells; nonproteolytic and generally nondiastatic; capable of utilizing paraffin.
  I. Initial mycelium fully developed, well branching, dividing into rods and generally into cocci.
    1. Substrate growth soft, without macroscopically visible aerial mycelium.
      a. Substrate mycelium yellow, orange, or red.
        a^1. Pathogenic.
          a^2. Substrate mycelium white, buff, or pale yellow.
            18. *Nocardia farcinica*
          b^2. Substrate mycelium yellow to red.
            6. *Nocardia asteroides*
        b^1. Not pathogenic.
          a^2. Paraffin decomposed.
            42. *Nocardia polychromogenes*
          b^2. Cellulose decomposed.
            13. *Nocardia cellulans*
      b. Substrate mycelium white to pink.
        a^1. Gelatin not liquefied.
          a^2. Growth on nutrient agar opaque, cream-colored.
            38. *Nocardia opaca*
          b^2. Growth on nutrient agar pink.
            10. *Nocardia calcarea*
            a^3. Aerial mycelium on milk white.
              31. *Nocardia leishmanii*
            b^3. Pellicle on milk pink.
              11. *Nocardia caprae*
            c^3. Pellicle on milk yellow.
              9. *Nocardia brasiliensis*
            d^3. Causing galls on blueberry plants.
              53. *Nocardia vaccinii*
        b^1. Gelatin liquefied.
          43. *Nocardia pulmonalis*
    2. Substrate mycelium hard, yellow.
      a. Aerial mycelium white; hyphae divides into chains of acid-fast cocci.
        40. *Nocardia paraffinae*
      b. Aerial mycelium not produced on organic media.
        41. *Nocardia petroleophila*
    3. Substrate growth cream colored, later becoming yellow.
      51. *Nocardia serophila*
    4. Substrate growth hard, orange-yellow.
      58. *Nocardia variabilis*
  II. Initial mycelium very short, rapidly dividing into rods and cocci.
    1. Growth pink.
      a. No cystites (swollen cells) formed.
        a^1. No indigotin from indole.
          16. *Nocardia corallina*
        b^1. Indigotin from indole.
          26. *Nocardia globerula*
      b. Cystites formed.
        49. *Nocardia salmonicolor*
    2. Growth coral-red.
      47. *Nocardia rubropertincta*
    3. Growth white, tan, or pink.
      a. No aerial mycelium.
        a^1. Growth tan.
          15. *Nocardia coeliaca*
        b^1. Growth white.
          28. *Nocardia intracellularis*
      b. Aerial mycelium produced.
        a^1. Growth frequently pinkish.
          53. *Nocardia transvalensis*
        b^1. Growth never pink.
          50. *Nocardia sebivorans*
    4. Produces no pigment, no growth on potato, coagulates milk.
      19. *Nocardia fastidiosa*

B. Nonacid-fast organisms with weakly refractive cells; no distinct formation of cocci; diastatic.
  I. Nonproteolytic, although some give gelatin liquefaction.
    1. Growth on agar pale cream.
      a. Gelatin not liquefied; starch hydrolyzed.
        35. *Nocardia mesenterica*
      b. Gelatin liquefied; starch not hydrolyzed.
        48. *Nocardia ruqosa*
    2. Growth on agar whitish.
      4. *Nocardia albicans*
    3. Growth on agar yellow.
      20. *Nocardia flava*
    4. Growth on agar green.
      59. *Nocardia viridis*
    5. Growth on agar yellow-green.
      14. *Nocardia citrea*

* This system was used, with certain minor omissions and additions, in the last edition of Bergey's Manual.

6. Growth initially colorless, producing a yellow-green pigment in 2 to 4 days.
   54. *Nocardia turbata*
7. Growth on agar dark brown and even black.
   a. No liquefaction of gelatin.
      37. *Nocardia nigra*
   b. Gelatin liquefied.
      29. *Nocardia ivorensis*
8. Growth consistency soft; aerial mycelium sparse.
   33. *Nocardia lutea*
9. Growth consistency medium; aerial mycelium profuse.
   8. *Nocardia blackwellii*
10. Growth cream-colored to pink; aerial spikes produced.
    52. *Nocardia sumatrae*
11. Growth grayish-yellow.
    36. *Nocardia muris*
12. Growth yellowish-orange.
    55. *Nocardia uniformis*
13. Pigment on protein media deep brown.
    44. *Nocardia rangoonensis*
14. Pigment on protein media light brown.
    12. *Nocardia caviae*

II. Proteolytic, although some are only weakly proteolytic.

1. Growth on nutrient agar with rapid formation of unbranched diphtheroid-like rods; no typical cystites; broth turbid.
   1. *Nocardia actinomorpha*
2. Growth white, shiny or pale.
   a. Dough-like consistency; breaks up into short rods.
      3. *Nocardia alba*
   b. Membranous, myceloid growth.
      32. *Nocardia listeri*
3. Growth on nutrient agar with extensive mycelium; simple unbranched rods not formed; cystites present. Broth clear.
   21. *Nocardia flavescens*
4. Growth cream-colored.
   a. Rapid liquefaction of gelatin.
      a¹. No aerial mycelium.
         25. *Nocardia gibsonii*
      b¹. Aerial mycelium scant, white.
         56. *Nocardia upcottii*
   b. Slow liquefaction of gelatin.
      17. *Nocardia dicksonii*
5. Growth rose-colored to bright red or red-orange.
   24. *Nocardia fructifera*
6. Growth pink to red.

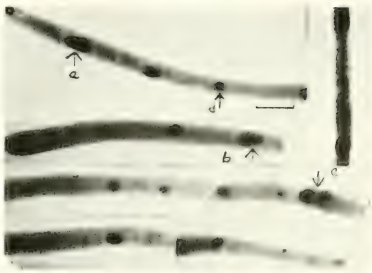

FIGURE 12. *N. rubra*, electron micrograph (Reproduced from: McClung, N. M. First Reg. Conf. Asia and Oceania, Tokyo, 1956).

   a. Gelatin not liquefied.
      2. *Nocardia africana*
   b. Gelatin slowly liquefied or not at all.
      46. *Nocardia rubra*
   c. Rapid liquefaction of gelatin.
      39. *Nocardia panjae*
7. Pigment on protein media light brown; color of growth pink.
   45. *Nocardia rhodnii*
8. Growth yellowish to golden brown.
   22. *Nocardia fordii*
9. Growth yellow to reddish-brown; soluble pigment brown to red.
   30. *Nocardia kuroishi*
10. Growth tan to buff-colored.
    23. *Nocardia formica*
11. Growth very limited on various media, except potato.
    27. *Nocardia hortonensis*
12. Occur in the sea; liquefy agar.
    a. Growth yellow.
       34. *Nocardia marina*
    b. Growth yellow-orange.
       7. *Nocardia atlantica*
13. Produce nodules on roots of plants.
    5. *Nocardia alni*

In addition to the species included in the above classification and described below, many more species of *Nocardia* have been recorded in the literature, either under this or under other generic names. Some are listed in Chapter 13, under the incompletely described forms. Others are synonyms. There is no question that some of the *Streptomyces* species described in Chapter 8 could just as

well have been included among the *Nocardia* forms. It is also possible some of those listed as *Nocardia* could just as readily have been included in the genus *Streptomyces*. Frequently, the decision of the investigator as to whether a certain culture should be included in one genus or another was perfectly arbitrary.

Descriptions of *Nocardia* Species*

1. *Nocardia actinomorpha* (Gray and Thornton, 1928) Waksman and Henrici, 1948 (Gray, P. and Thornton, H. Centrl. Bacteriol. Abt. II, **73:** 88, 1928).

Morphology: Growth colorless, smooth, consisting of long, branching filaments and rods, 0.5 to 0.8 by up to 10 μ. In older cultures, rods 2 to 3 μ long generally predominate. On some media, extensively branching hyphae occur. Not acid-fast.

Nutrient agar: Round colonies, 1 mm in diameter, convex, white, granular or resinous; long arborescent processes from the edge. No aerial mycelium.

Potato-glycerol agar: Growth dry, wrinkled, pink to orange.

Egg medium: Growth raised, dry, smooth, salmon-buff.

Gelatin: Colonies round, saucer-like, white, raised rim, edges burred. Liquefaction positive.

Nutrient broth: Turbid.

Milk: Coagulation and peptonization.

Starch: Hydrolyzed (diastase produced).

Sucrose: Inverted.

Nitrate reduction: Positive.

Phenol and naphthalene: Utilized.

Temperature: Optimum 25–30°C.

Source: Soil.

Remarks: Differs from *N. coeliaca* in liquefaction of gelatin. No acid from glucose, lactose, sucrose, or glycerol.

2. *Nocardia africana* Pijper and Pullinger, 1927 (Pijper, A. and Pullinger, B. D. J. Trop. Med. Hyg. **30:** 153–156, 1927).

Synonym: *Actinomyces africanus* (Pijper and Pullinger) Nannizzi Pollacci, 1934.

Morphology: Substrate growth consists of unicellular branching mycelium. Aerial mycelium sparse, consisting of short, straight hyphae. Not acid-fast.

Glucose agar: Colonies minute, red, discrete, round and piled up into a pale pink mass. Aerial mycelium thin, white.

Nutrient agar: Colonies discoid, flat, pink.

Glycerol agar: Growth made up of small, heaped-up, colorless masses with pink tinge; later, growth abundant, piled up, pale pink.

Potato agar: Growth bright red, made up of small, round colonies with colorless submerged margins, and piled up patches. Aerial mycelium stiff, sparse, white.

Egg medium: Colonies small, colorless, blister, partly confluent; becoming wrinkled, depressed into medium. Liquefaction slight.

Gelatin: Irregular pink flakes. No liquefaction.

Milk: Surface growth bright red. Medium gradually becomes opaque, reddish-purple, with slow peptonization.

Source: A case of mycetoma in South Africa.

3. *Nocardia alba* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, 1941, p. 1).

Morphology: Growth smooth or folded, made up of white colonies of a dough-like consistency; shiny or pale. Substrate mycelium breaks up into short rods 2.7 by 0.7 to 0.8 μ, later changing into a mass of coccus-like cells, 0.7 to 1 μ. Many cells are swollen, others form side buds. Not acid-fast. No aerial mycelium.

Synthetic agar: Inorganic salts used as sources of nitrogen; sugar, starch, or organic acids utilized as sources of carbon.

Nutrient agar: Good growth. No aerial mycelium.

* For further details concerning some of the species, the last edition of the Bergey Manual should be consulted.

Gelatin: Growth good. Positive liquefaction.

Milk: Coagulation and peptonization.

Starch: Rapid hydrolysis.

Cellulose: No growth.

Paraffin: No growth.

Nitrate reduction: Negative.

Sucrose: Inverted.

Source: Soil.

Remarks: Several subspecies were also listed: *N. chromogena*, *N. paulotropha* (*Actinobacillus paulotrophus* Beijerinck, 1914), *N. alba lactica*, *N. diastatica*, *N. hoffmanni*.

4. *Nocardia albicans* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk, SSSR, Moskau, 1941).

Morphology: Growth red, hyphae breaking up into rod-shaped cells, 12 to 25 by 0.6 to 0.7 μ, up to 50 μ in length. Cells straight or slightly curved, branching. Aerial mycelium not observed, except surface layer of sporophores, which produce a velvety appearance. Multiplication by fission, seldom by budding.

Nutrient agar: Growth good, smooth, shiny.

Gelatin: No liquefaction.

Milk: No change.

Starch: Hydrolyzed.

Cellulose: No growth.

Nutrient broth: Growth poor; produces faint turbidity, which settles on bottom and leaves a surface ring. No true mycelium. Cells rod-shaped 5 to 10 μ, seldom 15 to 20 μ.

Nitrate reduction: Negative.

Sucrose: Inverted.

Paraffin: Not utilized.

Source: Soil.

Remarks: Glycerol used as a source of carbon, and nitrate as a source of nitrogen.

5. *Nocardia alni* (Peklo *emend.* v. Plotho, 1941) Waksman (von Plotho, O. Arch. Mikrobiol. **12:** 1–18, 1941).

Morphology: Mycelium contains fatty globules; cells filiform, branching, disintegrating into short rods and cocci. Aerial mycelium usually absent, but may be formed on cultivation. Sporulating cultures form white, spherical to oval spores.

Agar media: Substrate growth compact, shiny, colorless or slightly brownish.

Gelatin: Surface pellicle. Liquefaction positive. Soluble pigment brownish.

Liquid media: Slimy surface film.

Tyrosine: Utilized as source of nitrogen; color turns red-brown.

Cellulose: Not utilized.

Carbon sources: Produces lactic acid from glucose and lactose.

Optimum reaction for growth: pH 6.0.

Habitat: Roots of the alder.

Remarks: Produces nodules on the roots of the host plant. Said to bring about nitrogen fixation in symbiotic culture with the plant.

6. *Nocardia asteroides* (Eppinger, 1891) Blanchard, 1895 *emend.* Gordon and Mihm, 1959 (Eppinger, H. L. Beitr. Pathol. Anat. **9:** 287, 1891; Blanchard, R. In Bouchard. Traite Pathol. Gen. **2:** 811, 1895; Gordon, R. E. and Mihm, J. M. J. Gen. Microbiol. **20:** 129, 1959).

Synonyms: *Cladothrix asteroides* Eppinger, 1890; *Streptothrix eppingeri* Rossi-Doria, 1891; *Actinomyces asteroides* Gasperini, 1892; *Oospora asteroides* Sauvageau and Radais, 1892; *N. asteroides* R. Blanchard, 1895. According to Ochoa and Sandoval (1956), *N. leishmanii* Chalmers and Christopherson, and *N. phenotolerans* Werkam and Gammel are synonyms of *N. asteroides*. According to Gordon and Mihm (1959), *N. caprae* (Silberschmidt) Waksman and Henrici, *N. eppingeri*, *N. minima*, and *N. sylvodorifera* are also synonyms.

Morphology: Typical actinomycete growth, usually yellow to orange to orange-red. Mycelium straight and fine; it breaks up into small, coccoid forms and rods. Some strains are acid-fast; others are only partially

so. Aerial hyphae produced; they vary from rudimentary to long branching. Some may produce chains of spores (Fig. 11).

Sucrose nitrate agar: Growth thin, spreading, orange. No aerial mycelium. No soluble pigment.

Peptone-beef extract agar: Growth much folded, light yellow, becoming deep yellow to yellowish-red. No soluble pigment.

Yeast-glucose agar: Growth flat to wrinkled, beige to dark pink. Some produce white aerial hyphae.

Potato: Growth much wrinkled, whitish, becoming yellow to almost brick-red.

Gelatin: Growth yellowish on surface. No liquefaction.

Milk: Orange-colored ring. No coagulation; no peptonization.

Starch agar: Growth restricted, scant, orange. No diastatic action.

Blood serum: No liquefaction.

Carbohydrate utilization: See Table 2.

Nitrate reduction: Positive.

Oxygen demand: Aerobic. According to Chalmers and Christopherson (1916), it may also grow anaerobically.

Temperature: Optimum 37°C. Some strains grow readily at 28°C.

Pathogenicity: Transmissible to rabbits and guinea pigs, but not to mice.

Source: Human infections and soil.

Remarks: A number of strains of acid-fast actinomycetes isolated from human lesions have deviated in certain particulars from the description of *N. asteroides*, but not sufficiently to warrant separation as different species. According to Gordon and Mihm, all strains of *N. asteroides* form whitish aerial hyphae, these varying from rudimentary to much branching. The following characteristics were considered the most valuable in the identification of the species: development of filamentous colonies with aerial hyphae; failure to hydrolyze casein and to dissolve the crystals of tyrosine and xanthine; acid production from glucose and glycerol; failure to form acid from arabinose, lactose, mannitol, inositol, and xylose; utilization of acetate, malate, propionate, pyruvate, and succinate, but not benzoate.

Numerous varieties of this species have been described. It is sufficient to mention *N. crateriformis*, *N. gypsoides*, and *N. pseudocarneus* (Gordon and Mihm, 1957).

Type culture: IMRU* 3308; also 504.

7. *Nocardia atlantica* (Humm and Shepard, 1946) Waksman (Humm, H. J. and Shepard, K. S. Duke Univ. Marine Sta. Bull. **3:** 78, 1946).

Synonym: *Proactinomyces atlanticus*.

Morphology: Hyphae long, branching, breaking up into rods and cocci, 0.5 to 0.7 μ. Involution forms in old cultures. Nonacid-fast. Aerial mycelium not produced.

Synthetic and organic media: Growth bright yellow or yellow-orange, smooth, compact, of a doughy consistency. Colonies flat with slightly raised center. Soluble pigments none. Mineral sources of nitrogen utilized.

Gelatin liquefaction: Positive.

Agar: Liquefied.

Milk: Coagulation rapid, acidified; peptonization slow.

Nitrate reduction: Positive.

Starch: Hydrolyzed.

Cellulose: Decomposed.

Chitin: Decomposed.

Agar: Slowly digested.

Alginic acid: Decomposed.

Carbon sources: Acid produced from arabinose, xylose, rhamnose, raffinose, fructose, galactose, sucrose, gum arabic. No acid from lactose, dulcitol, mannitol, or sorbitol. Organic acids utilized: gluconic, lactic, malonic. Organic acids not utilized: acetic, butyric, citric, etc.

Optimum temperature: 28–30°C.

Habitat: Marine algae and marine sediments.

* These designations represent the various culture collections where the type cultures are deposited.

Remarks: Another closely related culture has been described as *Proactinomyces flavus* (see *N. marina*).

8. *Nocardia blackwellii* (Erikson, 1935) Waksman and Henrici, 1948 (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 32–33, 1935).

Morphology: Growth consisting of short, rod-like filaments, growing out into longer sparsely branching hyphae. Aerial mycelium short, straight; frequently large, round or ovoid cells are interposed in the irregularly segmented chains of cells.

Glycerol nitrate agar: Growth extensive, granular, irregular, thin, pinkish.

Nutrient agar: Growth confluent, wrinkled, with small, round, pinkish, discrete colonies at margin.

Glucose nutrient agar: Growth abundant, pale pink, in form of small conical colonies, piled up, convoluted.

Potato agar: Colonies small, round, colorless. Aerial mycelium white. Later, colonies dull pink, submerged margins; few aerial spikes, moderate aerial mycelium at top of slant.

Gelatin: Colonies few, colorless, minute, along line of inoculation. Later, abundant, colorless colonies to 10 mm below surface; larger pink-yellow surface colonies with white aerial mycelium. No liquefaction.

Milk: Surface pellicle heavy, convoluted, bright yellow. No coagulation; no peptonization. Spalla states that milk is coagulated.

Carbon utilization: See Table 4.

Source: Hock joint of foal.

Type culture: ATCC 6846; NCTC 630.

9. *Nocardia brasiliensis* (Lindenberg, 1909) Castellani and Chalmers *emend.* Gordon and Mihm, 1959 (Lindenberg, A. Arch. Parasitol. **13:** 265–282, 1909; Castellani, A. and Chalmers, A. J. Manual of Tropical Medicine, 2d Ed. William Wood & Co., 1913, p. 816; Gordon, R. E. and Mihm, J. M. J. Gen. Microbiol. **20:** 129, 1959).

Synonyms: Gonzalez Ochoa (1945, 1953), Gonzalez Ochoa and Sandoval (1956), and Gordon and Mihm (1958) consider *N. brasiliensis* as the proper name for this organism. *A. mexicanus* Boyd and Crutchfield, *N. pretoriana* Pijper and Pullinger, and *N. transvalensis* Pijper and Pullinger, are considered as synonyms.

Morphology: Angularly branched filaments, bearing a few short straight aerial hyphae; later, growth becomes spreading and extensive. Aerial hyphae long and branching to short and gnarled; divide to form oval and cylindrical spores. Acid-fastness variable, from 100 per cent to none.

Glycerol nitrate agar: Growth in form of piled up pink mass. Aerial mycelium very scant, white, at margin.

Glucose nutrient agar: Colonies pale buff, umbilicated and piled up.

Yeast-glucose agar: Highly mutable. Growth yellow to yellowish-orange, finely wrinkled. Some strains produce no aerial hyphae; other strains form mat of whitish aerial hyphae. A few strains form amber to brown soluble pigment.

Potato: Colonies small, raised, pale pink; plug and liquid discolored. Later, growth dull buff, dry and convoluted at base, round and zonate at top of slant. Aerial mycelium white.

Gelatin: A few colorless flakes. No liquefaction

Milk: Surface growth yellowish. Pale pink growth up the wall of the tube. Solid coagulum in 1 month; later, partly digested.

Egg medium: Colonies few, round, colorless in 3 days. Later, irregular, raised pink mass, warted appearance; moderate degree of liquefaction.

Serum agar: Growth raised, convoluted, slightly pinkish.

Source: A case of mycetoma of the chest wall in a South African native.

Pathogenicity: To guinea pigs and humans.

Remarks: According to Gordon and

Mihm, *N. brasiliensis* is distinguished from *N. asteroides* by positive decomposition of casein and tyrosine and by acid formation from inositol and mannitol. Additional characteristics of this species include the following: xanthine not decomposed; acid produced from glucose and glycerol; no acid from arabinose, lactose, maltose, xylose, and other sugars; utilizes acetate, citrate, malate, propionate, pyruvate, and succinate, but not benzoate.

According to Mariat (1958), *N. asteroides* is characterized by a lack of proteolytic activity; utilization of urea, $(NH_4)_2SO_4$ and KNO_3 as sources of nitrogen, not of nitrite. Glucose, fructose, glycerol, and mannitol are utilized as sources of carbon, but not galactose, xylose, maltose, and starch, although paraffin is utilized.

N. brasiliensis is characterized by gelatin hydrolysis; utilization of urea, $(NH_4)_2SO_4$ and KNO_3 as nitrogen sources; utilization of glycerol, glucose, fructose, galactose, mannitol, xylose, and paraffin as carbon sources.

Type culture: IMRU 850.

10. *Nocardia calcarea* Metcalfe and Brown, 1957 (Metcalfe, G. and Brown, M. E. J. Gen. Microbiol. **17:** 568–569, 1957).

Morphology: Gram-positive and partially acid-fast. Mainly short rods (1.5 to 2.0 by 1.0 μ) together with unbranched aseptate filaments up to 10 μ in length and occasional branched filaments. Some filaments show lemon-shaped swellings. After 4 days, short rods show snapping division typical of corynebacteria; abundant unbranched filaments (5.0 to 8.0 by 1.0 μ) and a few branched filaments and cocci (1.0 μ) also present. Many rods show differentiation of a swollen spore-like structure; these are usually formed terminally or subterminally, one per rod. In the filaments they are often formed in chains. Colonies after 14 days consist of short rods, cocci and rods with swollen cells.

Agar media: Colonies circular, raised, soft, without aerial mycelium; pink or cream colored with distinct pink tinge when small.

Sucrose agar: Filaments are rare and short rods and cocci are the predominant forms throughout. Occasional lemon-shaped cells are formed on all media.

Glucose and mannitol agars: Very long branched filaments (10 to 25 μ) present after 2 days, often with terminal chains of swollen hyaline cells; these filaments usually fragment, but a few persist.

Yeast extract-peptone agar: Cycle shorter than on previous media, most of the filaments having fragmented into rods and cocci after 3 days.

Starch agar: Growth slight; no hydrolysis.

Milk: Heavy growth, turned alkaline; no peptonization.

Gelatin: Beaded growth at top of stab. No liquefaction.

Nitrate reduction: Positive.

Carbon utilization: Utilizes glucose, sucrose, and maltose; poor growth with lactose.

Paraffin: Growth heavy in basal salts medium with ammonium salt and flakes of paraffin wax.

11. *Nocardia caprae* (Silberschmidt, 1899) Waksman and Henrici, 1948 (Silberschmidt, W. Ann. inst. Pasteur **13:** 841–853, 1899).

Synonyms: This organism has been variously described as *S. caprae* (Price-Jones, 1901). *O. caprae* (Sartory, 1923), *A. caprae* (Nannizzi, 1934).

Morphology: Substrate growth forms thin, branching filaments, breaking up into rods. Aerial mycelium abundant on all media with tendency to form coherent spikes; mycelium not very polymorphous, but occasional thicker segments appear. Slightly acid-fast. Brownish soluble substance.

Glucose-peptone-beef extract agar: Growth irregular, bright pink, tending to be heaped up. Later abundant masses, frosted over with thin, white aerial mycelium.

Blood agar: Colonies minute, round, colorless, aggregated in broad pink zones. Aerial mycelium pale. No hemolysis.

Egg medium: Colonies few, colorless, some pink; aerial mycelium white. Later, growth

becoming dull pink, irregular, with scant white aerial mycelium.

Potato: Growth abundant. Aerial mycelium pale pink. Growth becomes membranous, considerably buckled.

Gelatin: Growth extensive, dull, with small raised patches of pink aerial mycelium; later, ribbon-like, depressed. No liquefaction.

Milk: Surface pellicle red. Solid coagulum; no peptonization.

Starch: No hydrolysis.

Source: Lesions in goats.

Pathogenicity: To rabbits, guinea pigs, and mice.

Remarks: According to Gordon and Mihm (1959) *N. caprae* is a synonym of *N. asteroides*; according to Schneidau and Shaffer (1957), however, the organism does not utilize paraffin and does not hemolyze blood, as shown on p. 68, Volume I.

Type culture: IMRU 783.

12. *Nocardia caviae* Snijders, 1924 (Snijders, Geneesk. Tijdschr. Ned. Indie **64:** 47, 75, 1924).

Morphology: Growth consists of initial segmented hyphae, producing elements of approximately even thickness, arranged in angular apposition; later, forms long, profusely ramifying threads with strongly refractile protoplasm. Aerial mycelium straight and branching, the sporophores forming occasional coiled tips, divided into cylindrical spores.

Glucose agar: Growth piled up, convoluted, cream-colored to pale pink. Aerial mycelium white.

Glycerol agar: Growth scanty.

Potato agar: Growth spreading, colorless. Aerial mycelium dense white.

Egg medium: Growth heavily corrugated, pale pink, with submerged margin. Aerial mycelium dense white. After 3 weeks, colorless transpired drops.

Potato: Colonies small, colorless. Aerial mycelium white, powdery. Later, abundant, raised, pale pink, confluent growth. Aerial mycelium white. Plug discolored.

Gelatin: A few colorless flakes. No liquefaction.

Milk: Surface growth colorless. Aerial mycelium white. Coagulation positive.

Nutrient broth: Surface pellicle cream-colored, wrinkled, extending up wall and breaking easily; moderate bottom growth, flaky. Medium discolored.

Source: Infected guinea pigs from Sumatra.

Remarks: Schneidau and Shaffer (1957) report that the organism is not acid-fast, grows at 46°C, utilizes paraffin, liquefies gelatin, hydrolyzes casein, liquefies starch, and shows positive hemolysis.

13. *Nocardia cellulans* Metcalfe and Brown, 1957 (Metcalfe, G. and Brown, M. E. J. Gen. Microbiol. **17:** 569–570, 1957).

Morphology: Gram-positive and partially acid-fast. Branching aseptate filaments, 30 to 40 μ in length, often with swellings at intervals; shorter filaments are less than 7.0 μ in length. Fragmentation commences about the fourth day, the number of short rods (1.5 to 2.0 by 1.0 μ) increasing rapidly. Hyaline spore-like structures are produced from the seventh day as slight terminal swellings on the filaments. After 28 days, the colonies consist of very short rods, cocci and spore-like cells.

Agar media: Colonies raised, soft, without aerial mycelium; cream-colored on most media but characteristically bright yellow on yeast extract-peptone agar.

Glucose agar: Filaments fragment less rapidly and are occasionally found after 28 days. Numerous Y-forms are found in older cultures.

Cellulose tubes: After 6 days there are long (20 to 30 μ) branched and unbranched filaments, many with terminal swellings. Fragmentation is rapid and short rods and cocci predominate during the stage of active cellulose decomposition. Old cultures are composed almost entirely of cocci.

Milk: Acid and curd produced.

Gelatin: Beaded growth at top of stab. No liquefaction.

Starch agar: Starch not hydrolyzed.

Nitrate reduction: Positive.

Carbon utilization: Glucose, sucrose and maltose utilized; acid produced.

Paraffin: Growth heavy with trace of yeast extract; no growth without yeast extract.

Type culture: ATCC 12,830.

14. *Nocardia citrea* (Krassilnikov, 1938) Waksman and Henrici, 1948 (Krassilnikov, N. A. Bull. Acad. Sci. USSR No. **1:** 139, 1938).

Morphology: Growth yellow to yellow-green, usually rough and folded, of a dough-like consistency. No soluble pigment. In young cultures, mycelium consists of very very fine threads 0.3 to 0.5 μ in diameter. After several days the cells break up into short rods 0.5 by 1.5 to 5 μ and into cocci 0.3 to 0.5 μ in diameter. Cells are the smallest of all the nocardias. Multiplies by fission and bud formation. No aerial mycelium. Not acid-fast.

Synthetic medium: Growth and pigmentation typical.

Nutrient agar: Growth good.

Gelatin: Liquefaction rapid.

Milk: Coagulation and peptonization.

Starch: Hydrolyzed rapidly.

Sucrose inversion: Positive.

Cellulose: No growth.

Fat: Weak growth.

Paraffin or wax: No growth.

Nitrate reduction: Positive.

Habitat: Soil and water.

15. *Nocardia coeliaca* (Gray and Thornton, 1928) Waksman and Henrici, 1948 (Gray, P., and Thornton, H. Centr. Bakteriol. Parasitenk. Abt. II, **73:** 88, 1928).

Morphology: Growth in form of short, curved, uneven-sided rods, 0.8 by 5 μ; occasional filaments up to 10 to 12 μ long; frequently beaded, occasionally swollen or branched; coccoid forms 0.8 to 1.2 μ in diameter are common, especially in older cultures. Not acid-fast, or occasionally slightly acid-fast.

Nutrient agar: Colonies less than 1 mm in diameter, round or irregular, raised, white, resinous, edge irregular, burred. Deep colonies irregularly round or oval, edge slightly broken. Slant filiform, convex, white, rugose, resinous, edge undulate.

Potato-glycerol agar: Growth dry, crumpled, orange-colored, becoming brown.

Gelatin: Surface colonies irregular, raised, white, rugose, dull edge entire. Deep colonies irregular, smooth or slightly broken. Stab convoluted, buff-white to yellowish, dull. Below surface the growth forms many irregular hollow lobes, giving a glistening appearance, to a depth of 3 to 4 mm.

Milk: Slightly alkaline after 5 to 7 days.

Nutrient broth: Turbid.

Nitrate reduction: None.

Phenol: Utilized.

Egg medium: Growth raised, smooth, moist, verrucose, buff-colored.

Temperature: Optimum 22–25°C.

Source: Soil.

Remarks: No acid from glucose, lactose, sucrose, or glycerol. No chromogenesis. Hollow lobes produced in deep gelatin cultures.

Type culture: ATCC 13181.

16. *Nocardia corallina* (Bergey *et al.*, 1923) Waksman and Henrici, 1948 (Hefferan, M. Centr. Bakteriol. Parasitenk. Abt. II, **11:** 459, 1904; Bergey *et al.*, Manual, 1st ed., 1923, p. 93).

Synonyms: *Nocardia minima* (*Proactinomyces minimus* Jensen). *Bacillus mycoides corallinus* Reader, 1926.

Morphology: Growth pink to red to orange-yellow. Branching mycelium, generally curved. In older cultures, hyphae degenerate generally into shorter rods and cocci. Not acid-fast.

Nutrient agar: Colonies smooth, pink, shining; border lighter, edge filamentous or with arborescent projections. As the colony grows, the cells in the interior break up into short rods and cocci which eventually form the mass of the colony. Cells on the outside remain filamentous, giving the colony a burr-like appearance, and often forming long arborescent processes. No soluble pigment.

Potato-glycerol agar: Growth filiform, raised, dry, wrinkled, yellowish-brown to coral red.

Gelatin: Surface colonies round, convex, smooth, pink, shining, edge filamentous; deep colonies, burrs. No liquefaction.

Milk: Reddish pellicle; milk becomes alkaline.

Nutrient broth: Usually turbid. Pink scum.

Paraffin and phenol: Utilized.

Nitrate reduction: Positive.

Starch: Not decomposed.

Sucrose: Not inverted.

Egg medium: Filiform, raised, dry, wrinkled, orange.

Temperature: Optimum 22–25°C.

Habitat: Soil.

Remarks: Some strains produce acid from glycerol and glucose. No acid or gas from sucrose, maltose, or lactose. Phenol and *m*-cresol are utilized. Some strains utilize naphthalene. Krassilnikov (1949) reports for his strains, good growth in high salt concentrations. Schneidau and Shaffer (1957) report positive acid-fastness, positive hemolysis and urease formation.

17. *Nocardia dicksonii* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council Spec. Rept. Ser. **17:** 203, 1935).

Morphology: Growth consists of long filaments, sometimes wavy. Aerial mycelium straight. Spores cylindrical.

Glycerol nitrate agar: Growth granular and wrinkled, cream-colored. Medium deeply discolored.

Glucose-asparagine agar: Growth wrinkled, colorless.

Potato agar: Growth abundant, colorless.

Egg medium: Growth yellowish-brown.

Starch agar: Strong hydrolysis.

Gelatin: Growth smooth, cream-colored on surface. Liquefaction limited.

Milk: Coagulated, peptonized.

Habitat: Unknown.

18. *Nocardia farcinica* (Nocard, 1888) Trevisan and De Toni, 1889 (Nocard, M. E. Ann. inst. Pasteur, **2:** 293, 1888; Trevisan, V., I. generi e le specie delle Batteriacee, Milan, 1889, p. 9).

Morphology: Growth yellow, of doughy consistency. Markedly acid-fast.

Nutrient agar: Colonies yellowish-white, irregular, refractive; mycelium filamentous.

Potato: Growth abundant, dull, crumpled, whitish-yellow.

Gelatin: Colonies small, circular, transparent, glistening. No liquefaction.

Milk: No coagulation; no peptonization.

Starch: No hydrolysis.

Nutrient broth: Clear, with granular sediment, often with gray pellicle.

Nitrate reduction: Negative.

Temperature: Optimum 37°C.

Pathogenicity: Pathogenic to certain domestic animals and guinea pigs.

Source: Cases of cattle farcy.

Type culture: IMRU 3318.

19. *Nocardia fastidiosa* Suter, 1951 (Suter, L. S. Mycologia **43:** 658–676, 1951).

Morphology: The organisms were similar on all media studied, showing in general a striking pleomorphism with coccoid, bacillary, and filamentous forms. Many of these were clubbed; some bore a striking resemblance to spermatozoa. Others were thick at one end and tapered down to filamentous tails. Filamentous forms were up to 25 μ in length and, not considering clubs or swollen portions, measured 0.2 to 1.2 μ in diameter, the average being about 0.8 to 1 μ. Stained

preparations never showed a richly branching character, but an alternate type of branching was fairly easy to demonstrate after about 7 days' incubation at 37°C. The coccoid forms were round, oval, or drop-shaped. Neither septa nor nuclei were seen. Spores were formed in short chains within mycelial strands and were of the same diameter as the mycelial strands. Similar spores were also found singly and extracellularly. The organisms taken from cultures were partially acid-fast.

Growth on agar media: Colonies were slow growing, appearing after 2 to 3 days as tiny specks, which after 7 days' incubation finally achieved, but never exceeded, a size of about 1 mm in diameter. To the naked eye they appeared grayish-white, compact, and smooth, and under low-power magnification they appeared fluffy, raised, compact at the center, and irregular and stringy at the edge, due to the presence of radiating and tangled filaments. Zigzag arrangements of elements and branches, clubs, and curls were seen at the periphery. The colonies were adherent to the medium. The top surface was dry and could be scraped off with a stiff wire loop, but neither the whole colony nor any part of it could be removed intact. On blood agar after 7 days' incubation, the colonies viewed by transmitted light showed a characteristic dense reddish center and a clear outer zone, both areas being very sharply defined.

Optimum temperature: 37°C.

Oxygen requirements: The organism is a facultative anaerobe, growing equally well in the presence or absence of oxygen.

Proteolytic activity: The organism is non-proteolytic. No odor of putrefaction was perceived in any of the cultures; gelatin was not liquefied; no growth occurred on serum plates or on coagulated human serum.

Gelatin stab: No growth after 28 days at 17–20°C.

Potato: No growth at 37°C.

Carbon sources: Acid formed from glucose; not from lactose, sucrose, maltose, or glycerol.

Nitrate reduction: Negative.

Habitat: Isolated from penile ulcer.

Remarks: *N. fastidiosa* is different from previously described species of *Nocardia* in the following ways: It is very fastidious in its growth requirements. It does not grow in synthetic media to which carbohydrates have been added; it will not utilize paraffin; it will not grow on potato or carrot; it will not grow on acid-maltose agar nor on acid-glucose agar; attempts to grow it on nutrient agar and on BHI agar have given variable results; its optimum temperature is 37°C. It is delicate and is relatively slow growing; it is never hardy or richly branching, and it does not produce a surface scum or a confluent or filiform growth. It is a facultative anaerobe, differing in this respect from all other *Nocardias* described with the exception of *N. farcinica* and *N. rubropertincta*.

The author summarizes its distinctive properties as follows: It produces a fairly compact colony composed of tangled mycelium and exhibits radiating, clubbed, branched, and curled elements at the periphery. Fragmentation of the mycelium and post-fission movement (zigzag arrangement) occur at the periphery of the colony. Arthrospores are produced. Stained preparations reveal partial fragmentation into bacillary and coccoid forms. Mycelial forms and spores average slightly less than 1 μ in diameter. Neither nuclei nor septa were observed. Branching is of an alternate type. It is partially acid-fast.

20. *Nocardia flava* (Krassilnikov, 1938) Waksman and Henrici, 1948 (Krassilnikov, N. A. Bull. Acad. Sci. USSR No. **1:** 139, 1938).

Not *Proactinomyces flavus* Humm and Shepard.

Morphology: Cells at first filamentous, 0.7 to 0.8 μ in diameter; later, they break into long rods and then into cocci 0.7 μ in

diameter. Some strains form chlamydospores. Numerous inflated cells of the bulbiform or fusiform type. Cell multiplication by fission, cross wall formation, rarely by budding. Not acid-fast.

Synthetic agar: Colonies bright yellow or golden.

Nutrient agar: Growth dirty, lustrous, or rough and folded, of a dough-like consistency, yellow to straw-colored. No soluble pigment.

Gelatin: No liquefaction.

Milk: No coagulation and no peptonization.

Starch hydrolysis: Slight.

Sucrose: Weak inversion.

Cellulose: No growth.

Paraffin and wax: No growth.

Fat: Weak growth.

Habitat: Soil.

21. *Nocardia flavescens* (Jensen, 1931) Waksman and Henrici, 1948 (Jensen, H. Proc. Linnean Soc. N. S. Wales **56:** 361, 1931).

Morphology: Substrate growth forms long, branched, nonseptate hyphae, 0.4 to 0.6 μ. On nutrient agar and potato, septa are formed, mycelium fragmenting, partly resembling highly branched mycobacteria. Aerial mycelium consists of fairly long hyphae of the same thickness as the vegetative hyphae, not very much branched, without spirals, often clinging together in wisps; hyphae break up into fragments of variable lengths, from 1.2 to 1.5 up to 10 to 13 μ, showing an irregular, granulated staining. Not acid-fast.

Nutrient agar: Substrate growth raised and much wrinkled, first dirty cream-colored, later dark yellowish-gray, of a soft, moist, curd-like consistency. Aerial mycelium absent. Soluble pigment absent.

Glucose agar: Substrate growth superficial, wrinkled, honey-yellow, of a hard and cartilaginous consistency. Aerial mycelium thin, smooth, white. Soluble pigment yellow.

Potato: Substrate growth much raised and wrinkled, first cream-colored, later yellowish-brown, soft and smeary. No aerial mycelium. No soluble pigment.

Gelatin: Liquefaction slow.

Milk: Coagulation; slow peptonization with acid reaction.

Starch: Hydrolyzed.

Cellulose: No growth.

Paraffin: No growth.

Sucrose: Inverted.

Glucose broth: Rather scant growth. Granulated, yellowish sediment; no surface growth. Broth clear. No pigment. No acidity.

Nitrate: Slight or no reduction.

Source: Soil.

22. *Nocardia fordii* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 15, 1935).

Morphology: Substrate growth consists of filaments of medium length. Aerial mycelium short, straight, sparse. Small oval spores on potato agar and starch agar.

Glycerol nitrate agar: Growth thin, extensive, golden brown, convoluted.

Nutrient agar: Colonies small, creamy to golden, ring-shaped; later, heaped-up patches, becoming golden brown and convoluted.

Egg medium: Colonies minute, cream-colored, elevated, becoming golden brown, raised.

Potato: Growth yellowish in thin terminal portion, tending to be piled up. Aerial mycelium scant, white, at top of slant. Later, growth abundant, golden brown, confluent, partly honeycombed, partly piled up.

Gelatin: No visible growth, slight softening of gelatin; later partial liquefaction.

Milk: Surface ring brownish. Coagulation positive.

Starch: Not hydrolyzed.

Source: Human spleen in a case of acholuric jaundice.

23. *Nocardia formica* Harris and Woodruff, 1953 (Harris, D. A. and Woodruff, H. B. Antibiotics Ann. 1953–1954, 609–614).

Morphology: Mycelial development extensive, with no fragmentation of hyphae. Ghost filaments and cytoplasmic condensations produced. In submerged culture, straight and curved rods develop, exhibiting the Y- and V-forms. Rods are 0.9 to 1.1 by 1.3 to 6.0 μ. Not acid-fast.

Sucrose nitrate agar: Growth very faint or none at all.

Glucose-asparagine agar: Growth fair. Aerial mycelium grayish-white. Sporulation poor.

Nutrient agar: Growth fair. Aerial mycelium none.

Peptone-glucose agar: Growth tannish-colored. Aerial mycelium white to grayish, gradually covering surface. Reverse side dark brown. Soluble pigment brown.

Egg medium: Growth excellent, buff-colored, convoluted, moist. No liquefaction. Medium not discolored.

Starch agar: Starch hydrolyzed.

Gelatin: Liquefaction rapid. Soluble pigment none. Growth settled on bottom of the tube.

Potato: Very poor growth.

Nitrate reduction: Positive.

Casein: Hydrolyzed.

Paraffin: Not utilized.

Optimum temperature: 28°C; good growth at 37°C.

Carbon utilization: No acid production in organic media from glucose, glycerol, lactose, maltose, and sucrose; acid produced in inorganic media from glucose, glycerol, lactose, maltose, but not from sucrose.

Antagonistic properties: Produces an antibiotic substance active against *Trichomonas* and swine influenza virus.

Source: Isolated from an abandoned nest of African ants in an imported mahogany log.

24. *Nocardia fructifera* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, 1941).

Morphology: Growth not compact, mostly of dough-like consistency, smooth or rough. Hyphae breaking up into rods and in some cultures into cocci. Not acid-fast. Aerial mycelium well developed, whitish to rose-colored. Sporophores long, straight or weakly wavy, but not spiral-shaped. Spores cylindrical, 1.5 by 0.7 μ.

Synthetic agar: Growth rose-colored to bright red. No soluble pigment.

Nutrient agar: Aerial mycelium weakly developed or absent entirely.

Gelatin: Liquefaction slow.

Milk: Coagulation positive; peptonization weak.

Sucrose: Inverted.

Starch: Hydrolysis weak.

Cellulose: Poor growth.

Paraffin: No growth.

Fats: Good growth.

Source: Soil.

Remarks: One strain was obtained as a mutant of another *Nocardia*; another strain was changed, after 8 months of cultivation, into a typical *Streptomyces*. This species is considered as a transition form between the two genera.

25. *Nocardia gibsonii* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 36, 1935).

Morphology: Young growing mycelium branches profusely at short intervals, finally grows out into long, frequently wavy filaments. Property of producing aerial mycelium apparently lost.

Nutrient agar: Colonies small, cream-colored, depressed, partly confluent, growing into an extensive wrinkled surface layer.

Glucose nutrient agar: Growth cream-colored, wrinkled, membranous.

Potato agar: Growth wrinkled, glistening, membranous.

Blood agar: Colonies small, discrete, yel-

lowish, irregularly wrinkled, clear hemolytic zone.

Egg medium: Colonies small, round, smooth, colorless, with conically elevated centers.

Potato: No growth.

Gelatin: Dull white flakes sinking as medium liquefies. Liquefaction rapid.

Milk: Coagulation positive; peptonization limited.

Starch: Not hydrolyzed.

Source: Human spleen in a case of acholuric jaundice. Injected into a monkey and reisolated.

Type culture: ATCC 6852.

26. *Nocardia globerula* (Gray, 1928) Waksman and Henrici, 1948 (Gray, P. Proc. Roy. Soc. (London) B **102:** 265, 1928).

Morphology: Growth orange to orange-buff. It consists of curved rods and filaments, 1 by 2 to 9 μ, with many coccoid cells, especially in old cultures. Rods and filaments frequently irregularly swollen. Not acid-fast. Capsules may be present.

Nutrient agar: Surface colonies irregularly round, 3 to 5 mm in diameter, convex, white, smooth, shining; edge undulate, erose. Deep colonies, lens-shaped.

Gelatin: Surface colonies irregularly round, 1 to 2 mm in diameter, convex, light buff, smooth, shining. Stab: nailhead, irregularly round, convex, pinkish-white, smooth, shining.

Potato-glycerol agar: Growth filiform, moist, smooth, pale pink.

Milk: Alkaline.

Nutrient and peptone broth: Turbid with viscous suspension.

Nitrate reduction: None.

Egg medium: Growth spreading, raised, moist, orange-colored.

Indole agar: Blue crystals of indigotin formed.

Temperature: Optimum 25–28°C.

Phenol: Utilized.

Source: Soil.

Remarks: This organism resembles most closely *N. corallina*. It is distinguished by producing a more watery type of surface growth, more nearly entire deep colonies, and more particularly by the production of indigotin from indole. No acid from glucose, lactose, maltose, sucrose, or glycerol.

Type culture: ATCC 13,130.

27. *Nocardia hortonensis* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 22, 1935).

Morphology: Substrate growth made up of very slowly developing unicellular mycelium, composed of long slender straight branching filaments. Aerial mycelium very sparse, forming straight hyphae only once on potato. Not acid-fast.

Glycerol nitrate agar: Colonies coiled, colorless, lustrous patches, isolated, with central depression.

Nutrient agar: Growth very slow, as few smooth, cream-colored, coiled colonies.

Glucose nutrient agar: Growth as coiled and heaped up cream-colored translucent masses.

Potato agar: Colonies colorless, blister; later dull green heaped and coiled mass. Medium becomes slightly discolored.

Potato: Colonies abundant, colorless, umbilicated, round, some coiled in raised masses; later, liberal olive-green growth. Aerial mycelium dense, velvety gray-green at top of slant.

Gelatin: Colonies round, cream-colored on surface and a few millimeters below. No liquefaction.

Milk: Surface growth green; peptonization positive. Color at first purple, later brown.

Source: From pus containing typical actinomycetic granules from parotid abscess.

28. *Nocardia intracellularis* Cutting and McCabe, 1949 (Cutting, J. T. and McCabe, A. B. Am. J. Pathol. **25:** 1–47, 1949).

Morphology: Filaments branched, becoming fragmented, composed of bacillary ele-

ments in series, 0.2 to 0.45 μ in width. Liquid cultures give branched colonies. The hyphae do not form club-shaped tips, and lack chlamydospores. Not discolored when stained with fuchsin and treated with acid alcohol.

Agar media: Colonies circular, raised, wet-shining, smooth, and nonmucoid.

Potato agar: No growth.

Gelatin: Growth poor. No liquefaction.

Glycerol broth: White, mucoid masses formed at bottom of tube. Medium remains clear.

Milk: Acid after 20 to 30 days.

Starch: Not changed.

Tyrosinase reaction: Absent.

Cellulose: Not decomposed.

Nitrate reduction: None.

Oxygen requirement: Does not develop in the absence of oxygen, but grows in an atmosphere having 10 per cent CO_2.

Paraffin: Used as the only source of carbon.

Temperature: Grows at 37.5°C and tolerates well temperatures up to 40°C.

Habitat: Observed in granuloma of infected lymph nodes and in the feces of a living patient whose death it ultimately caused. Observed also at autopsy in widely disseminated granulomatous lesions which it produced.

Type culture: ATCC 13,209.

29. *Nocardia ivorensis* Combes, Kauffmann and Vazart, 1957 (Combes, R., Kauffmann, J., and Vazart, B. Compt. rend. **224:** 821–824, 1582–1587, 1957).

Agar media: Substrate growth characterized by the black coloration of its coccoid bodies, by their elongation, by their resistance to dryness and to heat, by their cellulolytic properties, and by their production of an orange pigment on different media. Colonies at first whitish, centers becoming light orange to brown, later black. Black circles formed successively around the central circle and finally becoming confluent. Later, surface of colony is uniformly black, shiny, and waxy; at the periphery, grayish, radiant outgrowths develop in the agar, forming a more or less regular fringe; on the surface of this fringe brown, rapidly darkening, concentric zones appear.

Milk: Reddish-orange surface film, and isolated colonies adhering to the walls of the tube.

Potato: Growth orange, darkening in places, with the appearance of coccoid forms.

Gelatin: Growth scant, slow; later the culture is orange in color. Liquefaction positive.

Cellulose (filter paper or washed cotton), moistened with the synthetic medium: Light brown colonies appear in 2 days and turn dark at 6 days, being entirely composed of coccoid forms; later, colonies are entirely black. Cellulose progressively disintegrates.

Paraffin: White colonies appear at 5 days. They remain small and rapidly form coccoid elements.

Nitrate reduction: Positive.

Remarks: Three isolated cultures differed from each other mainly in the rapidity with which they formed coccoid elements. Organism closely related to *N. nigra*.

Habitat: Colony of termites on ivory coast of Africa.

30. *Nocardia kuroishi* Uesaka, 1952 (Uesaka, I. J. Antibiotics (Japan) **5:** 75–79, 1952).

Morphology: Aerial mycelium abundant. Sporophores slightly curved at first, later turning around each other. Acid-fast.

Glycerol nitrate agar: Growth thin, pale yellow. Aerial mycelium punctiform, white. Soluble pigment yellow.

Nutrient agar: Colonies wrinkled, grayish-yellow. No aerial mycelium. Soluble pigment faint grayish-brown.

Glucose nutrient agar: Growth abundant, at first yellowish-brown, then reddish-brown. Aerial mycelium scant, white at margin of

colonies. Soluble pigment red to wine-colored.

Potato: Growth moderate, at first red or brownish red, later dark brown. Aerial mycelium grayish white. Soluble pigment dark brown.

Glucose broth: Red colonies forming pellicle. Abundant, flocculent sediment. Soluble pigment dark brown.

Gelatin: Growth yellowish-brown, sinking into medium. No aerial mycelium. No liquefaction. Soluble pigment yellowish-brown.

Milk: No coagulation. Slow peptonization. Brown pigment.

Starch: Hydrolyzed.

Carbon source: Lactose well utilized.

Nitrate reduction: None.

Antagonistic properties: Produces an antibiotic neonocardin, active against various bacteria.

Source: Soil.

31. *Nocardia leishmanii* Chalmers and Christopherson, 1916 (Birt, C. and Leishman, W. B. J. Hyg. **2:** 120, 1902; Chalmers, A. and Christopherson, I. Ann. Trop. Med. Parasitol. **10:** 255, 1916).

Morphology: Initial cells frequently swollen, large and irregular, aggregated in short chains and then branching out into regular narrow filaments; later entire colonies asteroid in appearance, very fine and close angular branching, with aerial hyphae situated singly. Whitish-pink aerial mycelium generally abundant with irregularly cylindrical conidia. Acid-fast.

Glucose nutrient agar: Colonies rounded, elevated, red, with paler frosting of sparse aerial mycelium. No soluble pigment.

Glycerol agar: Colonies small, round, pink, tending to be umbilicated and piled up. Aerial spikes stiff, white.

Potato agar: Colonies minute, colorless, round. Aerial mycelium white, in patches.

Egg medium: Growth colorless, confluent, studded with little wart-like projections bearing stiff aerial spikes; later pinkish. Aerial mycelium white. Medium discolored.

Gelatin: Colonies small, pink. No liquefaction.

Milk: Surface growth; aerial mycelium white turning pink. Coagulum solid, later partly peptonized.

Pathogenicity: To rabbits, rats, and guinea pigs.

Source: Fatal case of lung disease and pericarditis in man.

Remarks: According to Gonzalez Ochoa and Sandoval (1956), *N. leishmanii* is a synonym of *N. asteroides*.

32. *Nocardia listeri* (Erikson, 1935) Waksmann (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 23–24, 1935).

Morphology: Sporophores short and straight. Spores oval.

Glycerol nitrate agar: Growth abundant, moist, cream-colored. Aerial mycelium powdery, white, with exuded drops.

Calcium malate agar: Growth poor, in form of a biscuit-colored membrane.

Nutrient agar: Growth smooth, moist, cream-colored, margin depressed, center elevated.

Glucose nutrient agar: Growth cream-colored, glistening.

Potato agar: Growth extensive, colorless, warted surface. Dirty pink coloration after 2 weeks. Scant white aerial mycelium after 4 months.

Potato: Growth abundant, dull, brownish, wrinkled. Aerial mycelium white.

Gelatin: Surface colonies round, white; after 45 days, confluent skin. Liquefaction slight.

Blood agar: Colonies small, round, cream-colored, with smooth, translucent surface. No hemolysis.

Serum agar: Colonies small, irregular, moist, cream-colored, tending to be heaped up; later somewhat transparent.

Milk: Coagulated. No change in reaction.

Source: From human material.

33. *Nocardia lutea* Christopherson and Archibald, 1918 (Christopherson, J. B. and Archibald, R. G. Lancet **2:** 847, 1918).*

Morphology: Growth consists of irregular, spreading, polymorphous colonies, comprising swollen and segmented cells of all shapes and sizes with markedly granular contents. Later cells more monomorphous, the filaments being arranged in angular apposition.

Glycerol nitrate agar: Growth in form of yellowish-pink, wrinkled membrane.

Nutrient agar: Growth abundant, coherent, moist, pink, membranous with round discrete colonies at margin.

Glucose nutrient agar: Growth scant, reddish, smeary.

Potato agar: Small filamentous colonies are formed; irregular angular branching. Aerial hyphae few, isolated, short, straight.

Potato: Growth carrot-red, moist, thick, granular in bands, partly raised and with discrete round colonies. Aerial mycelium sparse, colorless, very thin at top of slant.

Gelatin: Growth pale pink, wrinkled on wall of tube. Colorless punctiform and stellate colonies in medium. No liquefaction.

Milk: Growth orange-red on surface and at bottom.

Egg medium: Growth poor, dull pink.

Source: Actinomycosis of the lachrymal gland.

Remarks: According to Erikson, various saprophytes, such as *N. rubra* and *N. polychromogenes*, are closely related.

34. *Nocardia marina* (Krassilnikov, 1949) Waksman (Humm, H. J. and Shepard, K. S. Duke Univ. Marine Sta. Bull. **3:** 76, 1946; Krassilnikov, N. A., Guide to the identification of bacteria and actinomycetes, Moscow, 1949).

Synonyms: *Proactinomyces flavus* Humm and Shepard. *Proactinomyces citreus marinae* Krassilnikov.

Morphology: Growth smooth, bright yellow color, of a dough-like consistency. Hyphae long, filiform, branching, breaking down into short rods and cocci. No aerial mycelium.

Synthetic and protein salt water media: Good growth.

Gelatin liquefaction: Positive.

Agar: Liquefied.

Milk: No coagulation; peptonization positive.

Nitrate reduction: None.

Starch: Hydrolyzed.

Carbon sources: Acetic, lactic, and butyric acids utilized. Acid formed from various sugars.

Temperature: 25–30°C.

Habitat: Atlantic Ocean marine deposit.

35. *Nocardia mesenterica* (Orla-Jensen, 1919) Waksman and Henrici, 1948 (Orla-Jensen, S. The lactic acid bacteria, 1919, 181; Jensen, H. L. Proc. Linnean Soc. N. S. Wales **57:** 373, 1932).

Morphology: Growth forms extensive mycelium composed of richly branching hyphae of a somewhat variable thickness, 0.4 to 0.8 μ. No aerial mycelium. Later, hyphae divide into fragments of varying sizes and shapes, partly diphtheroid rods, but no real cocci. There is, particularly in complex organic media, a tendency to form large, swollen, fusiform to almost spherical cells, up to 3.5 μ in diameter. These may stain intensely with carbol fuchsin.

Glucose-asparagine agar: Growth fair, raised, granular, very pale yellow, glistening. Condensation water-clear.

Glucose-peptone agar: Growth excellent, spreading. At first flat and smooth, pale straw-yellow, perfectly hard and cartilaginous, later raised and strongly folded, of a loose, curd-like consistency, bright lemon-yellow.

Potato: Growth scant, restricted, soft, cream-colored smear.

Gelatin: Growth finely arborescent,

* Description after Erikson, D., Med. Res. Council Spec. Rept. Ser., **203:** 30, 1935.

cream-colored in the stab. Surface colony raised, folded, pale yellow. No liquefaction.

Milk: Small cream-colored granules along the tube. No proteolytic action.

Starch: Hydrolyzed.

Cellulose: Not utilized.

Nutrient broth: Good growth; voluminous, flaky, whitish sediment; broth clear.

Nitrate reduction: Negative.

Sucrose: Inverted.

Source: Fermented beets.

Remarks: Sodium nitrate, ammonium phosphate, and asparagine are utilized, although these are inferior to peptone as sources of nitrogen.

36. *Nocardia muris* (Schottmüller, 1914) de Mello and Pais, 1918 (de Mello and Pais. Arq. Hig. Pat. Exot. **6:** 183, 1918).

Synonyms: *Streptothrix muris-ratti* Schottmüller, 1914. *Streptobacillus moniliformis* Levaditi, 1925. *Actinomyces muris* Topley and Wilson, 1946. *Proactinomyces muris* Krassilnikov, 1941.

Morphology: Slender branching filaments, 0.4 to 0.6 μ in diameter, breaking up into rods and cocci. Often cells form long chains of bead-like cells, with terminal club-like swellings. Nonacid-fast.

Growth: None on ordinary media. Growth occurs in presence of serum, ascitic fluid or blood.

Nutrient agar: No growth.

Glucose agar: No growth.

Serum agar: Grayish-yellow, clear colonies, 0.2 to 0.3 mm in diameter, with smooth, glistening surface and entire edge. Easily emulsifiable.

Gelatin: No growth.

Potato: No growth.

Milk: No effect.

Nitrate reduction: None.

Blood agar: Like serum agar. No hemolysis.

Egg medium: Similar to growth on serum agar. No liquefaction.

Oxygen: Grows aerobically. Grows also under anaerobic conditions.

Acid production: Acid produced in serum agar media, with glucose and salicin, sometimes with maltose and lactose.

Habitat: Parasite inhabiting nasopharynx of rats. Isolated from body of patient bitten by a rat.

Remarks: Similar organisms by a variety of names, such as *A. putorii*, were also listed. Above description based on data of Topley and Wilson (1946).

37. *Nocardia nigra* (Krassilnikov, 1941) Waksman (not *N. nigra* Castellani and Chalmers; not *Streptothrix nigra* Rossi-Doria.) (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, 1941).

Morphology: Growth rough, folded, shiny, dough-like consistency. Cells thread-like, breaking up readily into rods 2 to 10 by 0.7 μ and cocci, 0.6 to 0.8 μ. No aerial mycelium. Gram-positive, not acid-fast.

Agar media: Growth poor, at first colorless or brownish, gradually becoming darker, later dark brown and even black. Pigment not excreted in medium. Many cells are swollen to 3 μ in diameter.

Potato: Growth good.

Gelatin: No growth. No liquefaction.

Milk: No change.

Cellulose: No growth.

Paraffin and wax: No growth.

Nutrient broth: Small sediment produced. Medium clear.

Carbon utilization: Utilizes glucose and mannose, with formation of acid.

Source: Seldom found in soil.

Remarks: Culture rapidly loses its viability on continued cultivation.

38. *Nocardia opaca* (den Dooren de Jong, 1927) Waksman and Henrici, 1948 (den Dooren de Jong, L. E. Centr. Bakteriol. Parasitenk. Abt. II, **71:** 216, 1927; Jensen, H. L. Proc. Linnean Soc. N. S. Wales **57:** 369, 1932).

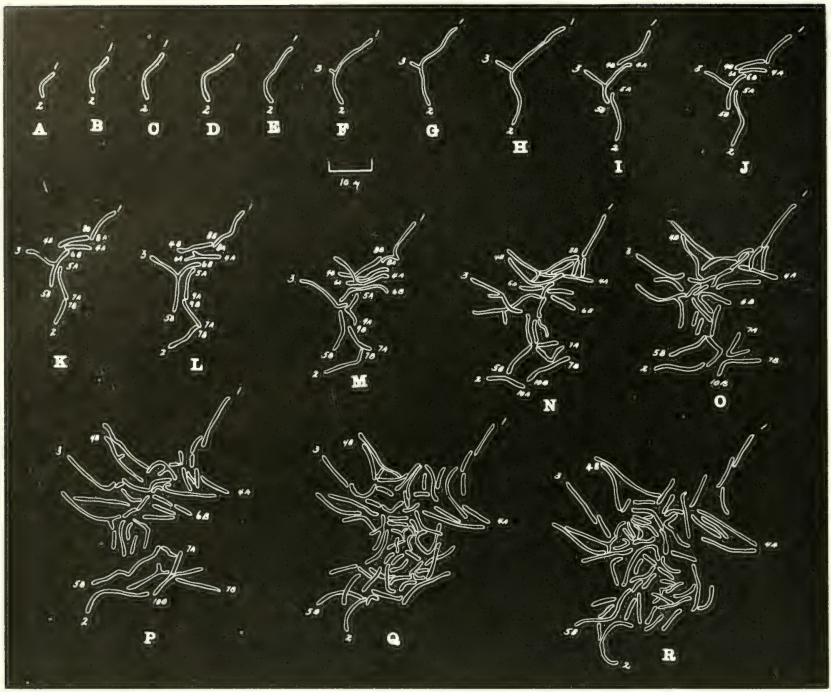

FIGURE 13. *N. opaca* (*N. erythropolis*), showing scheme of branching; glycerol nutrient agar, first sketch 10 hours incubation; others at hourly intervals (Reproduced from: McClung, N. M. Lloydia **12**: 153, 1949).

Synonyms: *Nocardia crystallophaga* (Gray and Thornton); *N. erythropolis* (Gray and Thornton); *Proactinomyces opacus* (Jensen).

Morphology: Growth lustrous, rose-colored to red. Hyphae long, curved, irregular and branching, breaking up into rods and cocci. Not acid-fast. Gram-positive (Fig. 13).

Potato-glycerol agar: Growth dry, rough, crumpled, pink to buff-colored.

Gelatin: Colonies round, convex, whitish, smooth, shining, with edges slightly arborescent. Stab: convex, whitish, smooth, resinous, filiform, erose. No liquefaction.

Egg medium: Growth spreading, smooth, moist, salmon-colored.

Potato: Growth covered with tufts of aerial hyphae.

Milk: Grayish pellicle. No coagulation, no peptonization. Reaction slightly alkaline.

Nitrate reduction: Positive.

Starch: Not hydrolyzed.

Sucrose inversion: Negative.

Carbon sources: Saturated, long chain aliphatic hydrocarbons are utilized as sources of energy.

Temperature: Optimum 30°C.

Source: Seldom found in soils.

Remarks: Differs from *N. corallina* and *N. polychromogenes* in that the cells are much longer than those of the former and

much shorter than those of the latter. Erikson (1949) added the following characteristics: Soft cream to pink growth on nutrient agar media. On synthetic media, growth colorless and thin, producing an initial mycelium, the hyphae dividing rapidly into short rods; addition of 0.01 per cent $MnSO_4$ stimulated production of pale pink pigment. Acid-fast cell elements predominated during periods of maximum growth and free air supply. A study of the morphology of *N. opaca* grown on hydrocarbons and fatty acids has been made by Webley (1955).

39. *Nocardia panjae* (Erikson) Waksman (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 1935, 16–17).

Morphology: Substrate growth made up of very small, round colonies; unicellular mycelium with slender, branching filaments. Aerial mycelium not visible on any medium, but occasional isolated aerial branches.

Glycerol nitrate agar: Growth poor; scant colorless patch.

Calcium malate agar: Growth colorless to pink, spreading; later, bright red mass, buckled and shining, colorless submerged margin.

Nutrient agar: Growth irregularly piled up, convoluted, colorless, easily detachable, brownish.

Glucose agar: Small colorless coiled mass, later heaped up as green growth.

Gelatin: Liquefaction rapid.

Milk: Surface growth pale green. Coagulation and peptonization.

Potato agar: Growth as small elevated, convoluted, colorless mass with purple tinge in center.

Egg medium: Colonies small, round, tough, colorless; margin well embedded. Later, colonies elevated, warted, darkened, medium discolored and broken. Slight degree of liquefaction; medium dark brown.

Source: An ulcer of the abdominal wall of a patient in India.

40. *Nocardia paraffinae* (Jensen, 1931) Waksman and Henrici, 1948 (Jensen, H. Proc. Linnean Soc. N. S. Wales **56:** 362, 1931).

Morphology: Growth hard, firm, yellowish, consisting initially of an extensive mycelium, with long, richly branching hyphae, 0.4 to 0.5 μ thick. After 5 to 6 days, numerous end branches swell to about double thickness, and divide into oval, spore-like elements, 0.8 to 1.0 by 1.2 to 1.5 μ. Division starts at the tips of the swollen branches and proceeds basipetally until most of the hyphae appear divided. Primary septa have not been seen in the hyphae. The spore-like elements are markedly acid-fast. Aerial mycelium white consisting of short, straight, not very much branched hyphae, 0.4 to 0.6 μ thick, which never show any differentiation into spores (see also Erikson, 1949).

Sucrose nitrate agar: Growth very scant, as thin colorless veil. Aerial mycelium trace, white.

Glucose-asparagine agar: Growth fair, flat, growing into medium; pale ocher-yellow to orange, with raised outgrowths on the surface. Aerial mycelium scant, white.

Nutrient agar: Growth slow, somewhat raised, ocher-yellow, hard, smeary surface loose. Aerial mycelium scant, small white tufts. No soluble pigment.

Potato: Growth mycelium granulated, first pale yellow, later deep ocher-yellow to orange. Aerial mycelium scant, white. No soluble pigment.

Gelatin: No liquefaction.

Milk: No coagulation; no peptonization.

Starch: No hydrolysis.

Cellulose: Not decomposed.

Paraffin: Readily utilized.

Nitrate reduction: Negative.

Sucrose: Not inverted.

Liquid media (milk, broth, synthetic solutions): Small, round granules of various yellow to orange colors, firm but can be crushed into a homogeneous smear. In old

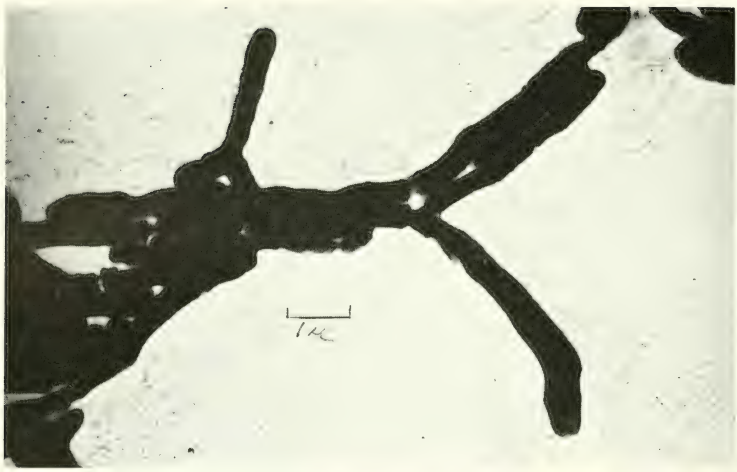

FIGURE 14. Ørskov's motile *Nocardia* (Reproduced by courtesy of N. M. McClung).

broth cultures, a thick, hard, orange to brownish surface pellicle is formed.

Habitat: Soil.

41. *Nocardia petroleophila* Hirsch and Engel, 1956 (Hirsch, P. and Engel, H. Ber. deut. bot. Gesell. **69:** 441–454, 1956).

Morphology: Grows slowly, but abundantly on all mineral media, faster in a petroleum atmosphere. It grows on certain organic media, but does not produce any aerial mycelium. Substrate mycelium breaks up readily into rods; "involution cells" are formed abundantly. Mycelial threads are long, monopodially branched, 0.6 to 1.2 μ in diameter, and contain "metacleromatic granules," readily stained with aqueous methylene blue. Aerial hyphae have the same diameter as the substrate hyphae, little branched. Aerial mycelium wets with difficulty. No aerial spores. Mycelial segments 1.2 to 5.0 by 1.2 to 12.5 μ. Gram-positive; not acid-fast.

Glucose-asparagine agar: Growth limited. Colonies 0.3 mm, white, yellow reverse. Aerial mycelium snow-white. Soluble pigment none.

Calcium malate agar: Growth limited.

Nutrient agar: Growth limited. Colonies whitish-yellow, 0.5 mm. No aerial mycelium.

Starch agar: Growth limited. Aerial mycelium snow-white; reverse yellowish. Starch not hydrolyzed.

Potato: No growth.

Gelatin: Growth in form of microscopic colonies. No liquefaction. No pigmentation.

Milk: Growth limited, yellowish, dry. No aerial mycelium. No coagulation; no peptonization.

Temperature: Optimum 25–28°C. Resistant to drying.

Salt concentration: Resistant to high concentration.

Habitat: Soil.

42. *Nocardia polychromogenes* (Vallée, 1903) Waksman and Henrici, 1948 (Vallée, H. Ann. inst. Pasteur **17:** 288–292, 1903; Jensen, H. Proc. Linnean Soc. N.S. Wales **56:** 79, 363, 1931).

Morphology: Growth bright red, coral-red to red-pink, of a doughy consistency, later becoming leathery. Aerial mycelium whitish with pink hue. Substrate growth

forms long wavy filaments, 0.4 to 0.5 by 70 to 100 μ, extensively branched but without septa. Older cultures consist entirely of rods, 4 to 10 μ, frequently in V-, Y-, or smaller coccoid forms. Gram-positive, not acid-fast, frequently showing bands and granules.

Nutrient agar: Growth scant, orange-red. No aerial mycelium. No soluble pigment.

Glucose agar: Growth raised, flat, glistening, rose-colored, later becoming folded and coral-red.

Gelatin: Growth along stab thin, yellowish, with thin radiating filaments. Surface growth flat, wrinkled, red. No liquefaction.

Milk: Growth starts as small orange-colored surface granules, later forming a thick, soft, orange-colored sediment. No coagulation; no peptonization.

Starch: Hydrolyzed.

Paraffin: Utilized.

Cellulose: No growth.

Temperature: Optimum 22–25°C.

Source: Blood of a horse; soil in France and Australia.

Remarks: Differs from *N. corallina* in the formation of very long filaments and in filiform growth in gelatin stabs.

Type culture: IMRU 3409.

43. *Nocardia pulmonalis* (Burnett, 1909) Waksman and Henrici, 1948 (Burnett, S. H. Ann. Rept. N. Y. State Vet. Coll. 1909–1910, 167).

Morphology: Mycelium acid-fast, especially in early stages of growth; breaks up readily into oval-shaped cells. Growth lemon-yellow with white aerial mycelium. Consistency of colonies leathery.

Peptone-beef extract agar: Growth moist, raised, in form of small, spherical colonies.

Glucose-peptone-beef extract agar: Growth dull, whitish, convoluted.

Potato: Growth abundant, in form of small, translucent, round colonies, becoming lemon-yellow. Later, growth becomes convoluted or folded with chalky white aerial mycelium. Color of plug brownish.

Gelatin: Colonies small, whitish, spherical; edges of colony becoming chalky white. Limited liquefaction.

Milk: Colonies on surface of the medium. Coagulation and gradual peptonization.

Pathogenicity: Nonpathogenic for rabbits and guinea pigs.

Source: Lungs of a cow.

44. *Nocardia rangoonensis* (Erikson, 1935) Waksman and Henrici, 1948 (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 33–34, 1935).

Morphology: Growth consists of branching hyphae which segment and present slipping and angular arrangement. Aerial hyphae few, short, straight, later developing into a profusely branching, long, waving aerial mycelium. Not acid-fast.

Glycerol nitrate agar: Growth dull, mealy, pink, wrinkled. Aerial mycelium scant, white. Medium slightly discolored.

Nutrient agar: Colonies round, lobate, umbilicated, raised, cream-colored to pale pink. Later, colonies colorless, medium discolored dark brown.

Glucose nutrient agar: Growth convoluted, coherent, cream-colored; medium discolored. Later, growth wrinkled, biscuit-colored, colorless margin. Aerial mycelium on border, white. Soluble pigment dark brown.

Potato agar: Colonies small, round, lemon-colored, partly confluent. Submerged growth greenish. Aerial mycelium white. Medium colored light brown.

Egg medium: Growth extensive, colorless. Aerial mycelium in center, pale pink.

Gelatin: Colonies abundant, minute in medium; larger, cream-colored colonies on surface. Aerial mycelium white. Brown pigment surrounding growth. No liquefaction.

Milk: Surface ring yellow. Coagulation positive; peptonization partial. Soluble pigment dark brown.

Source: Human pulmonary case of streptothricosis.

45. *Nocardia rhodnii* (Erikson, 1935) Waksman and Henrici, 1948 (Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 29, 1935).

Morphology: Substrate growth made up of minute colonies, composed of hyphal segments arranged in angular apposition. Aerial mycelium is short and straight. Later, growth becomes extensive and spreading, made up partly of long, branching filaments, and partly of short segments exhibiting slipping branching, each giving rise to aerial hyphae. Angular branching very marked, delicate, spreading, herringbone patterns being formed.

Sucrose nitrate agar: Colonies minute, colorless, round.

Glucose-asparagine agar: Growth abundant, coral-pink, convoluted, piled up.

Glycerol agar: Growth made up of dull pink colonies, round and umbilicated, becoming piled up and deeper coral-red.

Potato agar: Growth abundant, pink, piled up and stiff. Aerial mycelium white at top of slant.

Egg medium: Membrane salmon-pink, granular.

Gelatin: Colonies pale pink, in form of surface pellicle and as sediment. Liquefaction rapid.

Milk: Growth bright orange. Medium unchanged.

Nutrient broth: Salmon-pink flakes in sediment and colonies on surface. Medium discolored.

Source: From reduviid bug, *Rhodnius prolixus.*

Type culture: IMRU 653.

46. *Nocardia rubra* (*Actinomyces ruber sterilis* Krassilnikov, 1949) Waksman (Krassilnikov, N. A. Guide to the identification of bacteria and actinomycetes. Moskau, 1949).

Agar media: Growth red, smooth, nodular, slightly lustrous with a gravel-like appearance. No aerial mycelium produced under laboratory conditions. Most strains form no soluble pigment. Some produce a brownish substance. Slightly acid-fast (Fig. 12).

Milk: Unchanged.

Krassilnikov examined 25 different strains said to belong to this type, but differing from one another in intensity of color and in certain physiological properties. He believed that under certain conditions of growth these cultures would develop an aerial mycelium and proper sporulation.

This group was divided by Krassilnikov into four subgroups:

a. Flat, compact colonies, red to pink in color, pigment insoluble. Gelatin not liquefied, milk unchanged or only peptonized; starch not decomposed; nitrate not reduced.

b. Colonies raised, dry, crumbling at contact with loop; red to brownish-red in color. Ready growth on synthetic media. Gelatin liquefied slowly; milk coagulated slightly or only peptonized; nitrate reduced to nitrite; ready growth in paraffin and fats.

c. Colonies compact, growing deep into substrate; pink to light red in color; brown substance excreted into substrate. Gelatin slightly liquefied; milk peptonized by some strains, starch slightly decomposed; nitrates not reduced to nitrites. Some cultures grow slowly in cellulose. Do not grow on paraffin.

d. Flat or nodular colonies, growing compactly into medium. Frequently develop coremia on the surface; these consist of thickly interwoven sterile hyphae. The cultures grow poorly on artificial media. Gelatin not liquefied or only slowly; milk not changed or only slightly peptonized; starch not decomposed. No growth on cellulose. Ready growth on fats, paraffin, and wax.

Remarks: Krassilnikov considers this group to comprise transition forms between *Streptomyces* and *Nocardia*. *N. corallina* is believed to be a related form; a number of synonyms are listed such as *N. agrestis*, *N.*

minima, and others. According to Schneidau and Shaffer (1957), this form does not produce urease, whereas *N. corallina* does.

47. *Nocardia rubropertincta* (Hefferan, 1904) Waksman and Henrici, 1948 (Grassberger, R. Münch. med. Wochschr. **46:** 343, 1899; Hefferan, M. Centr. Bakteriol. Parasitenk. Abt. II, **11:** 460, 1904).

Morphology: Growth in form of small rods 0.3 to 0.9 by 1.5 to 3.0 μ, showing angular arrangement; later, nearly coccoid, 0.6 by 0.8 μ. Tendency for branching on glycerol agar, but branching does not occur commonly, though granules of aerial mycelium are sometimes seen. Not acid-fast or variable.

Nutrient agar: Colonies small, granular, becoming pink to red, depending on composition of agar.

Potato: Growth slow but excellent, intensive red, becoming dull orange.

Gelatin: Colonies irregular with crenate margin and folded surface, coral-red. Growth in stab at first thin, then granular to arborescent with chromogenesis. No liquefaction.

Milk: Surface scales thick, fragile, dull coral-red; also sediment. Milk becomes somewhat viscid after 3 to 4 weeks.

Nutrient broth: Faint uniform turbidity with salmon-pink pellicle, which is renewed on surface as it settles to form a red sediment.

Nitrate reduction: None.

Carbon sources: Utilizes benzene, petroleum, paraffin oil, and paraffin.

Temperature: Grows well between 20 and 37°C.

Oxygen requirement: Aerobic to facultatively anaerobic.

Source: Isolated from butter, soil, and contaminants of tuberculin flasks.

Remarks: *Mycobacterium*-like colonies with coral to vermillion-red chromogenesis on various media.

48. *Nocardia rugosa* DiMarco and Spalla, 1959 (DiMarco, A. and Spalla, C. Lab. Ricerche Farmitalia, Milano, 1959).

Morphology: Hyphae short, 0.6 to 0.8 μ in diameter, wavy, later angular, radiating from a center. After 20 to 24 hours, they break up into rods 8 to 20 μ long. No aerial mycelium. Nonacid-fast.

Glucose-asparagine agar: Growth colorless, raised, moist, wrinkled.

Glycerol agar: Abundant, lichenoid growth, dull cream. No consistency. Reddish-brown soluble pigment after 15 days.

Nutrient agar: Thick, cream-colored pellicle, rough and folded. Dough-like consistency. After 15 days, brown soluble pigment.

Potato agar: Growth smooth, folded, with wrinkled and lichenoid portions. Colorless. Soft consistency.

Milk: Coagulation; no peptonization.

Gelatin: Liquefaction positive.

Nitrate reduction: Negative.

Starch: Nondiastatic.

Sugar utilization: See Table 4.

Optimum temperature: 34°C.

Remarks: Nonpathogenic. Produces vitamin B_{12}.

Habitat: Cattle rumen.

Type culture: IMRU 3760.

This species was described further by Spalla (1959) as follows: It produces a colorless growth on glycerol, glucose, asparagine, and N-Z-amine agars. Acid is produced from glucose, galactose, ribose, rhamnose, *l*-arabinose, glycerol, *d*-mannitol, and adonitol; but not from *d*-mannose, sucrose, maltose, lactose, trehalose, raffinose, inulin, *d*-sorbitol, inositol, dulcitol, or salicin. Nitrate is not reduced. Starch is not hydrolyzed. Gelatin is liquefied. Milk is coagulated. The organism will resist a temperature of 60°C for 1½ hours, but not 3 hours. It is gram-positive. The terminal fragments are 1.56 ± 0.266 μ. No aerial mycelium is produced.

49. *Nocardia salmonicolor* (den Dooren de

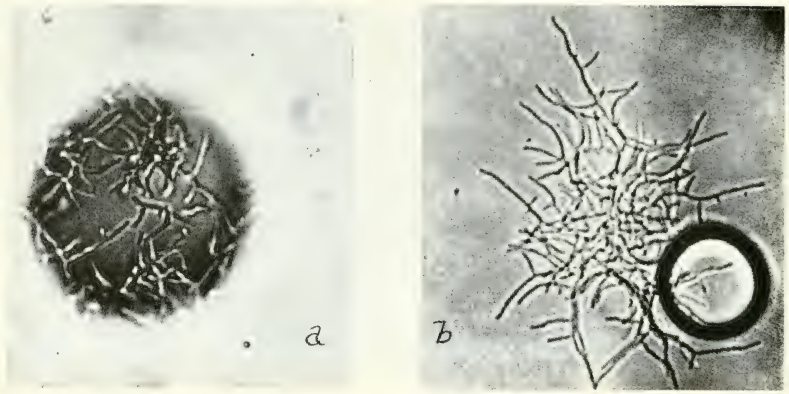

FIGURE 15. *N. salmonicolor*, growing on a hanging microdrop of liquid paraffin (a) surrounded by sucrose nitrate salt solution; (b) same plus 1.5 per cent agar (Reproduced from: Webley, D. M. J. Gen. Microbiol. **8**: 71, 1953).

Jong, 1927) Waksman and Henrici, 1948 (den Dooren de Jong. Centr. Bakteriol. Parasitenk. Abt. II, **71**: 216, 1927).

Morphology: Growth made up of short mycelium disintegrating into rods and cocci. Aerial mycelium sometimes stretching into quite long filaments, with small refractive granules. Many cells at the edge of the colonies show club- or pear-shaped swellings, up to 2.5 to 3.0 μ in width; many of these swollen cells later germinate with the formation of two more slender sprouts. Acid-fastness is found among the earlier stages of growth, especially in some of the strains and on some media (Fig 15).

Glucose-asparagine agar: Growth restricted, rather flat, edges lobate, surface warty, glistening; at first pale orange, later ocher-yellow; consistency crumbly.

Glucose-peptone-beef extract agar: Growth excellent, of a doughy consistency, spreading, flat, dense, edges lobate, surface folded, glistening, yellow, gradually changing to salmon-pink and deep orange-red.

Potato: Growth good, raised, warty, crumbly, glistening, at first buff, changing to orange, and finally to almost blood-red.

Gelatin: Growth in stab scant, arborescent. Surface colonies small, wrinkled, orange. No liquefaction.

Milk: Pellicle of small cream-colored granules, later a thick orange sediment. No coagulation and no peptonization, although milk appears slightly cleared, the reaction becoming alkaline.

Starch: Not hydrolyzed.

Sucrose: Not inverted, although readily utilized with sodium nitrate as a source of nitrogen.

Paraffin: Readily utilized as a source of carbon.

Cellulose: No growth.

Phenol: Not utilized.

Remarks: A detailed study of the acid-fast properties of this species has been made by Erikson (1949). It closely resembles *N. corallina*.

50. *Nocardia sebivorans* Erikson, 1954 (Erikson, D. J. Pathol. Bacteriol. **68**: 387–393, 1954).

Morphology: Gram-positive, partially acid-fast. Aerial mycelium white, with characteristic nonwetting properties. Both substrate and aerial mycelium show spon-

taneous segmentation into shorter and longer cells of coccoid or bacillary dimensions.

Agar media: Colonies firmly attached to medium. No soluble pigment. No acid produced from glucose, mannitol, lactose, sucrose, starch, raffinose, galactose, rhamnose, sorbitol, maltose, dulcitol, glycogen, or glycerol.

Sucrose nitrate agar: Growth fair; nitrate utilized.

Gelatin: No liquefaction.

Carbon utilization: Paraffin is good source of energy, also *n*-dodecane. Cresols not utilized.

Temperature: Can withstand exposure to 90°C for 10 minutes in a phosphate buffer suspension; denser suspensions withstood 3 minutes at 100°C (Erikson, 1955).

Habitat: Pus in a case of empyema.

Pathogenicity: Rabbits and guinea pigs, slightly for mice.

Remarks: Cells have avidity for oily substances (lipophilic). Under unfavorable conditions of growth, on the surface of solid paraffin, large clubs and hexagonal cells are produced (Erikson, 1955).

Type culture: NCTC 8595.

51. *Nocardia serophila* (Sartory and Bailly, 1947) *emend.* Waksman (Sartory, A. and Bailly, C. Compt. rend. **224:** 1533–1534, 1947).

Morphology: Hyphae produce angular growth, much branched, occasionally curved, 0.4 to 0.5 μ in diameter. Nonmotile. Rarely certain secondary branches are spiral shaped. Terminal and intracellular arthrospores. Gram-positive. Acid and alcohol resistant.

Growth characteristics: Grows with difficulty on ordinary solid or liquid media; grows well on serum or blood media.

Liquid peptone and serum media: Nonviscous, cream-colored growth, detaches from tube by agitation, medium remaining clear.

Coagulated serum media: Growth in form of small colonies, white at first, later becoming yellowish cream-colored.

Biochemical properties: Indole negative, H_2S positive, neutral red not reduced. Sugars like glucose, sucrose, lactose, galactose, and mannose not attached.

Milk: Coagulation positive; peptonization positive.

Coagulated serum: Not liquefied.

Nitrate reduction: Positive.

Oxygen demand: Strict aerobe.

Optimum temperature: 35–37°C.

Pathogenicity: Pathogenic to guinea pigs and rabbits.

Habitat: Isolated from urine of patient suspected of renal tuberculosis.

52. *Nocardia sumatrae* (Snijders *emend.* Erikson) (*N. cuniculi*, Snijders, Geneesk. Tijdsch. Ned. Indie **64:** 47, 75, 1924).

Not *Streptothrix cuniculi* Schmorl, 1891; not *Nocardia cuniculi* de Mello, 1919.

Morphology: Growth made up of large swollen cells, giving rise to ramifying filaments or to small chains of short, thick segments which branch out into more regular hyphae. Sometimes the irregular elements are beset with spiny processes before giving rise to typical long, branching filaments. Later the picture becomes more monomorphous, and short straight aerial hyphae are borne, which presently exhibit irregular segmentation.

Glycerol nitrate agar: Colonies small, round, elevated, cream-colored, margins depressed; becoming smooth, discrete, yellowish.

Glucose-asparagine agar: Colonies minute, colorless, becoming dull pink, partly confluent and piled up. Aerial spikes few, stiff, pink

Nutrient agar: Colonies small, round, elevated, cream-colored, umbilicated and radially wrinkled.

Egg medium: Growth scant, pinkish, smeary.

Potato: Growth coral-pink, dry, granular,

covered to a considerable extent with white aerial mycelium; piled up in center, discrete colonies at margin.

Gelatin: Few flakes. No liquefaction.

Milk: Heavy yellow growth attached to walls; solid coagulum in 1 month.

Nutrient broth: Surface colonies cream-colored, scale-like; abundant, flocculent bottom growth.

Source: Infected rabbits.

Remarks: Description given after Erikson (1935).

53. *Nocardia transvalensis* Pijper and Pullinger, 1927 (Pijper, A. and Pullinger, B. D. J. Trop. Med. Hyg. **30:** 153, 1927).

Morphology: Initial growth made up of unicellular hyphae, the central branch being frequently broader and showing dense granular refractile contents. Aerial mycelium white, forming straight hyphae, in some cases becoming clustered into irregular spikes. Colorless drops are exuded and a pink coloration is produced in the densest part of the growth on synthetic glycerol agar. Angular branching with division of substrate filaments. Aerial hyphae irregularly segmented. Acid-fast.

Glycerol nitrate agar: Growth in form of small, pink coiled masses. Aerial mycelium thin, white.

Nutrient agar: No growth.

Glucose nutrient agar: Colonies raised, granular, pink. Aerial mycelium white.

Potato: Growth dry, raised, convoluted, pink. Aerial mycelium white.

Gelatin: Growth poor, in form of a few irregular, colorless flakes. No liquefaction.

Milk: No change.

Starch: Not hydrolyzed.

Egg medium: Growth in form of small, irregularly raised, coiled, dull pink mass.

Source: A case of mycetoma of the foot, in South Africa.

Pathogenicity: To guinea pigs.

Remarks: According to Gonzalez Ochoa and Sandoval (1956), *N. transvalensis* is a synonym of *N. brasiliensis*.

54. *Nocardia turbata* Erikson, 1954 (Erikson, D. J. Gen. Microbiol. **11:** 198–208, 1954).

Morphology: Typical actinomycete, producing a fine mycelium composed of slender filaments, 0.1 μ in diameter, which fragment into rods and coccoid cells. Under appropriate conditions, many cells are motile. Nonacid-fast.

Agar media: Growth good. Colonies small, 0.1 to 2.0 μ. Initially colorless, later producing a yellow-green pigment on nutrient agar. Pigment production favored by free air supply, suppressed by acid reaction.

Broth cultures: Turbid when young; sedimentation of cells later, when pellicle and clarification of medium produced.

Acid production: Positive with glucose, sucrose, maltose, lactose, galactose, xylose, arabinose, glycerol, starch; negative with mannitol, raffinose, rhamnose, sorbitol, dulcitol (using a casein hydrolysate medium).

Oxygen demand: Aerobic.

Optimum temperature: 20–30°C.

Nitrate: Utilized.

Gelatin: No hydrolysis, except in presence of peptone (slowly).

Paraffin utilization: Negative.

Habitat: Probably soil.

55. *Nocardia uniformis* Marton and Szabo, 1959 (Marton, M. and Szabo, I. Acta Microbiol. Acad. Sci. Hung. **6:** 131–135, 1959).

Morphology: The filaments of the substrate mycelium rapidly break up into rods and less frequently into coccoid bodies. The size of these forms is 0.7 to 1.1 μ by 1.1 to 4.0 μ. In old cultures, swollen, club- or bottle-shaped forms appear. The hyphae of the slightly developed aerial mycelium are straight or waved, nonseptate, and contain

oval oidiospores. The mycelium is gram-positive and is not acid-fast.

Agar media: The strains give nonbutyrous colonies growing into the agar, with moderately striated dull surface covered with slightly developed white powder-like aerial mycelium. The color of the colonies is a constant yellowish-orange; it never turns red or yellow; no soluble pigment is produced. In liquid synthetic media a surface pellicle resembling agar colonies is formed.

Gelatin: No liquefaction.

Milk: No coagulation; no peptonization.

Sugar inversion: None.

Starch hydrolysis: None.

Nitrate reduction: Rapid.

Paraffin utilization: Slight or none.

Optimum temperature: 14–37°C.

Carbon utilization: Does not utilize mannose, dextrin, inulin.

Habitat: Deep layers B_1 horizon of saline soils.

56. *Nocardia upcottii* (Erikson, 1935) Waksman (Gibson, A. G. J. Pathol. Bacteriol. **23:** 357, 1920; Erikson, D. Med. Research Council Spec. Rept. Ser. **203:** 22–23, 1935).

Morphology: Growth forms long, straight filaments, much interwoven and ramified. Aerial mycelium slight, transient, slightly acid-fast.

Glycerol nitrate agar: Colonies small, round, cream-colored, glistening; heavy texture, margins submerged. Later, growth very much convoluted and raised, broad submerged margin; medium becomes slightly reddish.

Calcium malate agar: Growth limited, colorless, membranous, with undulating margin.

Nutrient agar: Colonies smooth, shining, round, cream-colored; margin submerged. Aerial mycelium scant, white. Later, colonies are large with greenish tinge; very sparse aerial mycelium gradually disappears.

Glucose nutrient agar: Colonies smooth, round, cream-colored; margin depressed, centers elevated, hollow on reverse side; later a coherent membranous growth, yellowish.

Potato agar: Growth poor, in form of small, colorless, blister colonies. Medium slightly discolored.

Egg medium: Colonies round, flat, colorless, scale-like, some marked by concentric rings and slightly hollowed in center. Growth becomes yellow-brown.

Blood agar: Colonies large, drab, heavily textured. No aerial mycelium. No hemolysis.

Gelatin: Growth abundant, flocculent, cream-colored on surface. Gradual liquefaction.

Source: From the spleen in a case of acholuric jaundice.

57. *Nocardia vaccinii* Demarec and Smith, 1952 (Demarec, J. B. and Smith, N. R. Phytopathology **42:** 249–252, 1952).

Morphology: Growth in form of rods and filaments, 0.4 μ to 0.8 μ in diameter; granular appearance when stained; eventually breaking up into bacillary forms. Few cells acid-fast. Fat demonstrated by staining with Sudan black B.

Sucrose nitrate agar: Growth scant, gray.

Nutrient agar: Growth poor, slow, granular, gray, sometimes pinkish in old cultures.

Gelatin: Growth dry, ribbon-like. No liquefaction.

Starch nutrient agar: Growth dry, ribbon-like, pinkish to orange. Hydrolysis of starch positive.

Potato: Growth slow, spreading, raised, gray.

Milk: Growth dry, raised, gray with orange spots. No peptonization.

Nitrate reduction: Positive.

Carbon utilization: With ammonia as the source of nitrogen, acid formed from glucose, sucrose, glycerol, and mannitol; reactions variable with arabinose and xylose; no growth on lactose or sorbitol.

Paraffin: Utilized.

Temperature: Growth best at 25–28°C; inhibited at 32°C; none or very scant at 37°C.

Antibiotic activity: None.

Habitat: Causes formation of bud-proliferating galls on blueberry plants.

Type culture: ATCC 11,092.

58. *Nocardia variabilis* (Cohn, 1913) Waksman (Cohn, T. Centr. Bakteriol. Parasitenk. Orig. **70:** 290–306, 1913).

Morphology: Cells initially filamentous, breaking up into rods and cocci. Nonacid-fast.

Agar media: Colonies round, smooth and lustrous, sometimes nodular; light brownish in color to orange-yellow. Colonies attached fast to the agar and partly removed with some effort.

Gelatin: Growth orange-yellow. No liquefaction.

Milk: Surface pellicle gradually becoming light orange. No coagulation; no peptonization.

Broth: Colorless surface pellicle, readily dropping to bottom. Medium remains clear.

Temperature: Optimum 37°C; good growth at 42°C; weak growth at 45°C.

Potato: Growth thin, colorless, becoming in time yellow to orange-red; finally brown.

Blood media: No hemolysis.

Oxygen demand: Markedly aerobic.

Habitat: Isolated from bladder of cystitis cases in man. Pathogenic to guinea pigs.

Remarks: Said to be similar to *A. ochroleucus*, *A. ochraceus*, and *A. carneus* of Neukirch (1902). According to Krassilnikov (1949), it is closely related to *N. africana*.

59. *Nocardia viridis* (Krassilnikov, 1938) Waksman and Henrici, 1948 (Krassilnikov, N. A. Bull. Acad. Sci. USSR. No. **1:** 139, 1938; Guide to the identification of bacteria and actinomycetes. Moskau, 1949).

Morphology: Growth dark green in color. Colonies of doughy consistency on certain media (wort agar, potato), and compact on others (nutrient). Pigment insoluble in medium and in organic solvents. On protein media, cells develop to form a thin mycelium without visible cross walls. Cells often branching, 0.7 to 0.8 μ in diameter, with cross wall. After 5 to 7 days the cells break up into rods 5 to 15 μ long. Cocci not observed. Cells multiply by fission, seldom by budding. No aerial mycelium. Gram-positive. Not acid-fast.

Nutrient agar: Growth compact. Thin mycelium produced.

Potato: Growth rough, much folded.

Gelatin liquefaction: Slow or none.

Milk: No coagulation; no peptonization; some reports of positive peptonization.

Starch: Not hydrolyzed. Spalla (1939) reported positive hydrolysis.

Sucrose: Not inverted.

Nitrate reduction: None.

Paraffin and fats: Growth good; less on wax.

Cellulose: No growth.

Habitat: Soil.

Davis and Freer (1960) described as a new species *N. salivae*, an aerobic actinomycete isolated regularly from the human mouth. Strains of this species are characterized by their saccharolytic power, which thus distinguishes them from the typical soil nocardias (see also von Magnus, 1947; Howell *et al.*, 1959). Hirsch (1960) described *N. saturnea*, an aerobic organism occurring in dust and capable of utilizing petroleum.

Chapter 4

Characterization of *Streptomyces* Species

Important Characters to be Considered for Recognition of Species and Varieties of *Streptomyces*

In the identification and characterization of *Streptomyces* species, the following characters should be considered:

1. Morphological properties:
   (a) Structure of substrate mycelium.
   (b) Nature and formation of aerial mycelium.
   (c) Structure and branching of sporophores.
   (d) Size and shape of spores.
   (e) Surface of spores.
2. Cultural properties on various media:
   (a) Growth characteristics.
   (b) Development of aerial mycelium.
   (c) Color of aerial and substrate mycelium.
3. Biochemical properties:
   (a) Production of soluble pigments in organic and in inorganic media.
   (b) Utilization of carbon sources.
   (c) Starch hydrolysis.
   (d) Sucrose inversion.
   (e) Cellulose decomposition.
   (f) Proteolytic activities: liquefaction of gelatin, blood serum, and casein; coagulation and peptonization of milk.
   (g) Utilization of nitrogenous compounds.
   (h) Formation of oxidases: tyrosinase and laccase.
   (i) Reductases: nitrate reductase, sulfate reductase.
   (j) Formation of antibiotics and vitamins.
   (k) Formation of H_2S in peptone-iron agar.
4. Sensitivity to antibiotics:
   (a) Sensitivity to pure antibiotic preparations.
   (b) Phenomena of "cross-resistance" and "cross-sensitivity" on artificial media.
5. Sensitivity to phages.
6. Serological reactions.
7. Chemical composition.
8. Ecological properties.
9. Genetic relationships.
10. Age of culture. Information should be submitted concerning the age of the culture when the particular properties were studied and the manner in which the culture has been kept in the laboratory.
11. Type cultures. The culture should be deposited in a recognized collection and the assigned number reported. Every possible means for preservation of the culture should be used. Preservation of strains by lyophilization, soil culture, mineral oil seals on active slants, or storage in deep freeze is believed to reduce physiological changes to a minimum. With the lyophilization technique within the reach of even the small laboratory, there is no excuse for an investigator, particularly one publishing on designated strains, to "lose" his strains.

In characterizing *Streptomyces* species, only certain media should be used and well-

defined conditions of growth recognized. Unnecessary media and nonessential details had better be left out to avoid cumbersome descriptions and nonduplicable characteristics that may apply to varieties or strains rather than to species. A larger number of media and more detailed descriptions may not only fail to give additional information but may complicate the description of the species to such an extent as to render the identification of freshly isolated cultures difficult.

The composition of the media is usually given first consideration for descriptive purposes. According to Waksman (1958), Shinobu (1958), and others, these media should include: (a) at least three synthetic media, preferably sucrose-sodium-nitrate-salt or sucrose-ammonium-salt agar, glucose- or glycerol-asparagine agar, and calcium malate or calcium citrate agar; (b) two or possibly three organic media, such as nutrient (peptone-beef extract) agar, yeast extract-glucose agar, potato-glycerol-glutamate agar, or oatmeal agar; (c) three or four complex natural media, notably potato plugs, gelatin, and milk; (d) peptone-iron-yeast extract agar for H_2S production; (e) tyrosine medium for the tyrosinase reaction; and (f) a synthetic medium for carbohydrate utilization.

Very few, if any, other media are required. Liquid media, with the exception of those previously listed, are better left out.

Morphological Properties

The method of study of the morphological properties of the streptomycetes would include visual microscopic examination *versus* electron microscopic studies; direct examination *versus* study of stained preparations; and hanging drop *versus* agar surface cultures.

STRUCTURE OF SUBSTRATE MYCELIUM

The substrate mycelium of a *Streptomyces* does not, as a rule, segment spontaneously into bacillary or coccoid forms. It produces leathery or tough-textured growth, remaining nonseptate and coherent even in old cultures. Although no true septa are observed in young cultures, it has recently been reported that older cultures show at least occasional septation. The compactness of this substrate growth is responsible for the fact that liquid media are always clear, unless the culture has been subject to phage or lytic action.

NATURE AND PROPERTIES OF AERIAL MYCELIUM

The aerial mycelium is usually thicker than the substrate mycelium. While the morphology of the substrate mycelium is usually undifferentiated, the aerial mycelium of streptomycetes, under fixed conditions of culture, shows sufficient differentiation that a miscellaneous assortment of isolates can be segregated into a number of groups having like morphological characteristics. This is one of the most important criteria for classification in the genus *Streptomyces*. Several aspects relating to the aerial mycelium may be considered:

a. Gross macroscopic appearance. The relative abundance, structure (cottony, velvety, powdery), formation of rings or concentric zones, and pigmentation of the aerial mycelium are important diagnostic criteria.

b. Microscopic properties. The microscopic structure of the aerial mycelium gives a clear picture of the morphology and reproductive structures of the organism. The hyphae may be long or short, with extensive or little branching. The branching may be simple or complex, monopodial or sympodial, broom-shaped or verticillate. The fruiting bodies or sporophores are short or long, occurring singly, in clusters, or as verticils; they are straight, wavy, or spiral-forming. The spirals or coils are either long and open or short and compact. Spiral formation may take place on one medium and not on others.

FIGURE 16. Schematic representation of different types of spirals produced by various *Streptomyces* species; the spirals range from long to short, from compact to irregular (Reproduced from: Shinobu, R. Mem. Osaka Univ. Lib. Arts and Ed. B. Nat. Sci. 7, 1958).

Before a culture is pronounced as forming no spirals, therefore, it must be grown on a variety of selective media that will allow optimum sporulation. Drechsler (1919) suggested use of the right-hand or left-hand curvature of the spirals as a diagnostic feature, but this, too, is influenced by the composition of the medium (Ettlinger *et al.*, 1958; Shinobu, 1958). Verticil formation is also an important characteristic of certain species; it can be simple or branching (primary or secondary verticils), the branches being straight or forming spirals; but this property as well is influenced to some extent by the composition of the medium. Although nocardiae may produce sporulating aerial filaments, these are never spiral-shaped (Fig. 16).

Waksman (1940, 1950) divided the organisms belonging to the genus *Actinomyces* (largely the forms now included in the genus *Streptomyces*) into the following five subgroups on the basis of the structure of the sporulation hyphae.

I. Straight sporulating hyphae, monopodial branching, never producing regular spirals.

II. Spore-bearing hyphae arranged in clusters, or broom-shaped arising from compression of the sporophores.

III. Spiral formation in aerial mycelium; long, open spirals.

IV. Spiral formation in aerial mycelium; short, compact spirals.

V. Spore-bearing hyphae arranged on mycelium in whorls (verticils) or tufts.

Krassilnikov (1941, 1949) divided the genus *Actinomyces* (largely forms included in *Streptomyces*) on the basis of the following properties: (1) spiral forming *versus* straight sporophores; (2) alternate distribution of sporophores on aerial mycelium *versus* verticil formation; (3) spherical *versus* oval spores; (4) colorless *versus* pigmented cultures; (5) white *versus* colored aerial mycelium; (6) saprophytes *versus* parasites.

Aiso *et al.* (1948) divided the genus *Streptomyces* on the basis of the structure of the aerial mycelium into six types:

I. Spirals not formed.

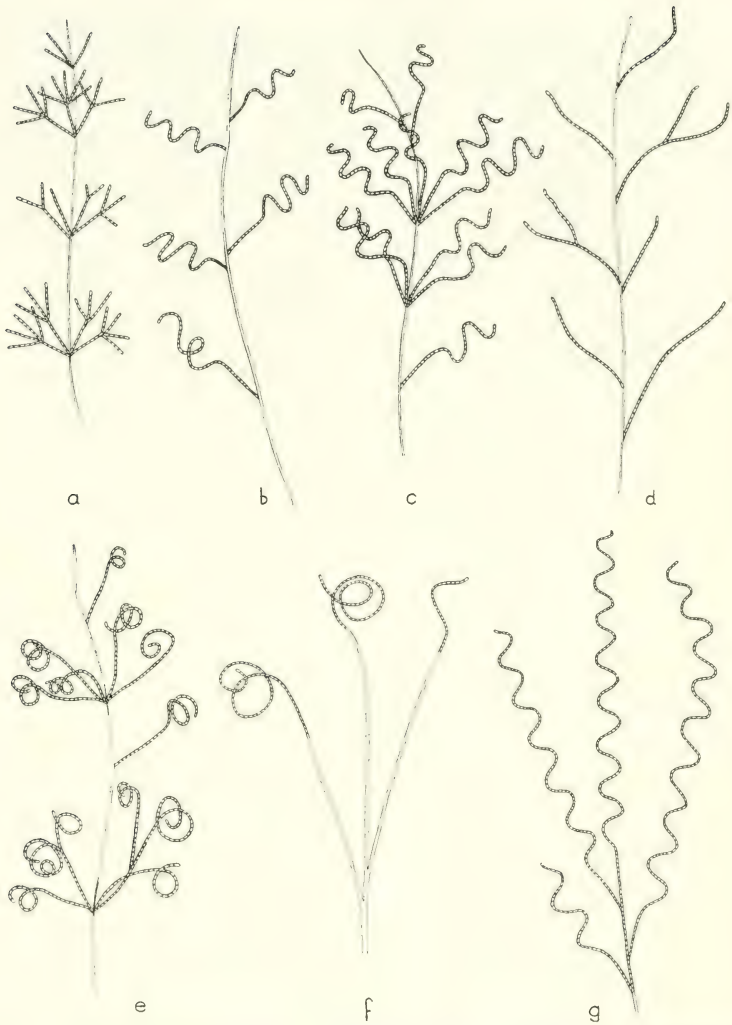

PLATE I. Diagrammatic representation of the morphology of the sporophores of *Streptomyces* (Reproduced by special permission from Ettlinger *et al.* Arch. Mikrobiol. **31**: 336, 1958).
a. Sporophores produce straight branching verticils on sterile aerial hyphae; *S. reticuli* type. b. Sporophores produce open spirals as side branches on sterile aerial hyphae; *S. purpurascens* type. c. Sporophores produce on sterile aerial hyphae verticils with open, more or less regular spirals; *S. noursei* type. d. Sporophores formed as side branches on sterile aerial hyphae, straight or slightly wavy; *S. phaeochromogenes*. e. Sporophores produced as verticils on sterile aerial hyphae, with open, irregular spirals; *S. echinatus*. f. Sporophores monopodially branched, forming irregular open spirals at the end of long hyphae; *S. lavendulae*. g. Sporophores monopodially branched, with open, regular spirals; *S. parvullus*.

PLATE II. Diagrammatic representation of the morphology of the sporophores of *Streptomyces* (Reproduced by special permission from Ettlinger *et al.* Arch. Mikrobiol. **31**: 337. 1958).
h. Sporophores produce numerous short, monopodial branches on sterile hyphae; *S. ramulosus*. i. Sporophores sympodially branched, forming tufts upon short main axes; *S. griseus*. k. Sporophores produced upon a long, straight main axis, monopodially branched with frequent, regular spirals; *S. fradiae*. l. Sporophores monopodially branched, straight or slightly wavy; *S. antibioticus*. m. Sporophores monopodially branched along the whole axis with open, irregular spirals; *S. erythraeus*. n. Sporophores monopodially branched, with narrow, compact spirals; *S. violaceoniger*. o. Sporophores sympodially branched, in the form of trees with a long main axis; *S. viridogenes*. p. Sporophores monopodially branched, stiff and straight; *S. venezuelae*.

1. Straight, very little branching.
2. Wavy, abundant branching.

II. Spirals formed.
1. Spirals formed on the axis, irregular branching.
2. Spirals formed on branches in clusters.

III. Verticils produced.
1. Verticillate branches entangled like a net.
2. Verticillate branches formed on both axis and branches, making primary and secondary verticils.

Okami (1952) grouped the genus *Streptomyces* on the basis of formation of aerial mycelium into the following types:

I. Spirals not formed.
1. Branches produced.
2. Branches not produced.

II. Spirals formed.
1. Spiral form mostly compact.
2. Spiral form mostly loose.

Shinobu (1958) criticized the systems of Aiso and Okami on the basis that insufficient attention was paid to the nature of the medium. In the system of Aiso *et al.* the distinction between 1 and 2 of each type appeared to him to be unclear, many forms belonging to an intermediate type. Okami's system was considered as incomplete because the formation of verticils was not taken into consideration. Waksman's system was believed to be comparatively better, but even this system was criticized because cluster or broom-shaped sporophore formation was not considered as a sufficient characteristic, and because a strain does not necessarily have only one kind of spiral, but usually forms various kinds of spirals which coexist (Pl. II).

Hesseltine *et al.* (1954) and Pridham *et al.* (1958) considered the sporophore morphology as reasonably stable under definite nutritional requirements of the organisms. Several morphological groups were suggested. The components of each group were considered as suggesting a logical natural arrangement. The physiological data can be used to produce "species" or "species-groups," with morphology as a starting point. Seven morphological sections were created as subdivisions of the genus *Streptomyces*. However, this system as well was considered by Shinobu as having certain defects because (a) culture media for morphological study were not examined thoroughly enough; and (b) some of the sections may often coexist in one strain.

Shinobu (1958) emphasized the following morphological properties of the aerial mycelium:

a. Outward appearance of mycelium (powdery, cottony, leathery).

b. Branching, especially the formation and nature of verticils.

c. Formation and nature of spirals.

d. Formation and shape of spores.

e. Thickness and length of mycelium.

Shinobu examined in detail the various properties of the aerial mycelium, emphasizing again the need for suitable synthetic media. He concluded that the nature of the aerial mycelium is one of the most important characteristics for taxonomic study, but that it should be considered in connection with composition of the medium. The aerial mycelium was classified into the following three groups, from the standpoint of branching and formation of spirals.

Group I. Straight or wavy aerial mycelium, monopodial branching, never producing spirals or clusters.

Group II. Spiral formation in the aerial mycelium; long or short; loose or compact; open or closed.

Group III. Verticil or cluster formation in the aerial mycelium.

The loss of ability to form aerial mycelium and sporogenous hyphae by certain *Streptomyces* cultures, on the one hand, and the formation of aerial mycelium and sporophores by certain species and strains of *Nocardia*, on the other, led Bradley (1959) to question the distinction between these two genera. We have here simply another

case of natural overlapping between man-made concepts of genera and species or the improper labeling of cultures. Gordon and Mihm (1957) emphasized that it is easy to understand how a culture of *N. asteroides* that formed acid-fast coccobacilli, rods, and short filaments and whose growth was heavy, finely to coarsely wrinkled, cream-colored to orange, and without noticeable aerial hyphae, could be mistaken for a *Mycobacterium*. A culture of *N. asteroides*, however, that produced nonacid-fast, long, tangled filaments and a cream-colored, pale yellow, or beige growth thickly covered with whitish aerial hyphae, could just as easily be accepted as a *Streptomyces*.

Numerous other studies have been made of the micromorphology of the various species and groups of *Streptomyces*, as in the work of Burkholder *et al.* (1954), Hesseltine *et al.* (1954), Ettlinger *et al.* (1958), Flaig and Kutzner (1960), and others.

c. Spores. The spores, also called conidia, produced from, or in, certain hyphae of the aerial mycelium, or the "sporogenous hyphae," may be oblong, oval, or spherical. Krassilnikov (1949) attached great importance to this character, as determined by the light microscope, as a diagnostic feature. Kriss *et al.* (1945) were the first to use the electron microscope for study of spores of *Streptomyces*. This was followed by the work of Carvajal (1946); Küster (1953); Flaig *et al.* (1952, 1955, 1958); Baldacci and Grein (1955); Grein (1955); Vernon (1955); and others. Flaig *et al.* (1952) found that the spores of some strains had smooth surfaces while others had spiny surfaces. They later detected spores with hairy and warty surfaces; the nature of the nitrogen source influenced the appearance of the spore surface, organic nitrogen favoring spine formation. Küster (1955) classified *Streptomyces* spores into two groups: (a) those producing a smooth surface and (b) those having a rough surface. Each of these groups was divided into three subgroups, based on shape of the spores. Thus there are spores with smooth surfaces, with spines, with hairs, or with warty protuberances, and spores that are globose, long-ovoid, and cylindrical.

On the basis of a system of classification that they had outlined, Baldacci and Grein (1955) examined 50 strains of streptomycetes with the electron microscope. Three types of spores were recognized: (1) Oval, more or less transparent spores; these were either smooth or rough, the latter having a spiny or hairy surface; the spines were either short and thick or long and thin. (2) Round, opaque spores, usually smooth. (3) Polyhedral spores, smooth and transparent, or slightly curved, wrinkled, and opaque. The form of the spores was constant for the series in Baldacci's system. It can hardly be used, however, as a species characteristic. A correlation was observed (Pridham, 1959) between spore characteristics and sporophore morphology (Table 6).

According to Preobrajenskaya *et al.* (1959, 1960), strains within one species as a rule have a similar type of spore surface. Cultures with a white, yellow, greenish-yellow, yellow-gray, pink, or lilac mycelium have smooth spores; those with a bluish aerial mycelium have spiny and hairy spores, and species with a gray aerial mycelium have spores of all types. The diagnostic value of spore surface characteristics was found to be dissimilar for the various sections. The correlation between the gray and bluish species and the character of the surface of the spores was considered as insignificant. Tresner *et al.* (1960) also emphasized the importance of spore surface in classifying species of *Streptomyces*; size and shape of spores of most species were considered of limited usefulness in taxonomic differentiation.

Lechevalier and Tikhonienko (1959) reported that the spores of *S. viridochromogenes* were mostly elongated and those of *S. violaceus*, spherical. The spines formed by

TABLE 6

*Morphology of sporophores and spores of streptomycetes** (Pridham, 1959)

Specific epithet	NRRL No.	Original strain no.	Sporophore morphology†	Spore morphology from electron micrograph
griseus	B-1598	Carvajal SL 842	Straight to flexuous (RF)	Smooth-walled
bikiniensis	B-1049	Waksman 3515	Straight to flexuous (RF)	Smooth-walled
canescus	2419	Com. Sol. 811.0	Straight to flexuous (RF)	Smooth-walled
venezuelae	B-902	Gottlieb 8-44	Straight to flexuous (RF)	Smooth-walled
cinnamonensis	B-1588	Okami 154-T3	Hooks and open loops (RA) with many straight (RF) sporophores	Smooth-walled
flavcolus	B-1334	ATCC 3319	Hooks and open loops (aberrant) (RA)	Spiny to hairy
albus	B-1685	Waksman (ATCC 618)	Spirals (aberrant) (S)	Smooth-walled
hygroscopicus	B-1865	NRRL isolate	Spirals (S)	Spiny
chartreusis	2287	Upjohn K-189	Spirals (S)	Spiny

* Data taken from Carvajal, 1946; Vernon, 1955. All other data on spore morphology supplied by K. L. Jones.

† Sporophore morphology determined according to the methods of Pridham *et al.*, 1958.

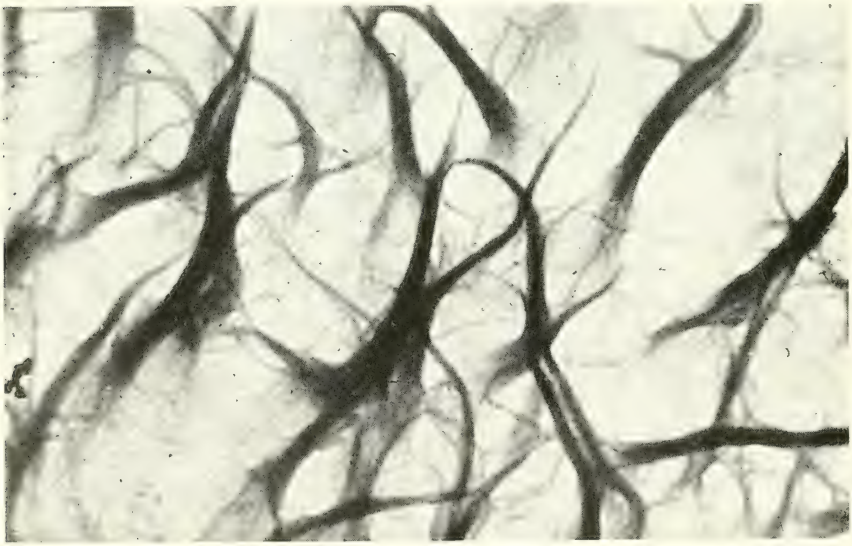

FIGURE 17. Coremia formation by certain *Streptomyces* species, × 500; stained by Corti's method (Courtesy of Dr. J. Giolitti, Milan, Italy).

strains of both species differed cytologically. The spines of the first seemed to be part of the cell wall, whereas the spines of the second seemed to be very superficial, appearing only on the envelope. It was concluded that spine formation is a stable characteristic of the spores. The shape of the spores varied with the composition of the medium. It was suggested that complex organic media be avoided for spore study.

COLONY STRUCTURE

The nature of the *Streptomyces* colony growing on a standard agar plate has been considered as among the important criteria for characterizing and recognizing a particular organism. One may question, however, the significance of this property in describing a species. The morphology of the colony, notably its general appearance, size, shape, and texture, can all be readily determined by superficial examination. Various other properties may be recognized from a study of the colony. Krainsky used the structure of the colony, especially its size and shape, as one of the major diagnostic criteria.

The superficial examination of gross colony structure, particularly its texture, can be of some help. Pridham* and others have noted the following very general correlations:

1. Straight to flexuous cultures generally are flat with a velvety, granular, or powdery texture.

2. Loop cultures generally are flat with a velvety to slightly granular texture.

3. Spiral cultures generally are elevated with a somewhat floccose texture. Occasionally, spiral cultures that are flat with a velvety or granular texture may be seen. In spiral cultures that are somewhat floccose the sporulating aerial mycelium often consists of long sterile hyphae with sporophores branched oppositely, singly, or sometimes in an apparent verticillate fashion.

* Personal communication.

FIGURE 18. Sclerotium formation in *Streptomyces* (Prepared by H. Lechevalier, Institute of Microbiology).

FIGURE 19. Sclerotium in species of *Streptomyces*, designated as new genus *Chainia* by Thirumalachar (Prepared by H. Lechevalier, Institute of Microbiology).

4. Verticillate cultures generally are elevated and floccose. Aberrant verticillate cultures generally are flat with a velvety texture.

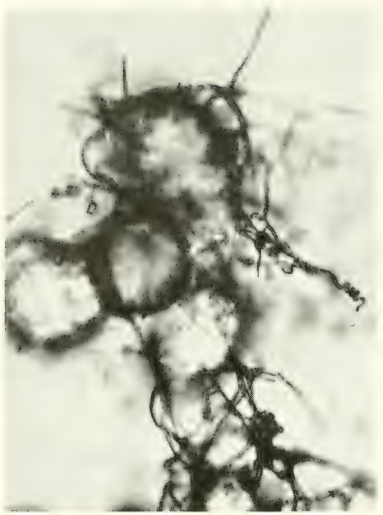

FIGURE 20. Sclerotium in species of *Streptomyces*, designated as new genus *Chainia* (Prepared by H. Lechevalier, Institute of Microbiology).

According to Krassilnikov (1955, 1959, 1960), the nature of the sporophore is a permanent property, being straight in the *S. globisporus* group, spiral-shaped in the *S. coelicolor* and *S. violaceus* groups. The same constancy is true of the shape of the spores, cylindrical *versus* oval or spherical, and of the manner of spore formation, namely, fragmentation *versus* segmentation. Branching of the sporophores, namely, vertical formation *versus* monopodial branching, is also a constant, although a variable, property. No single property, however, is sufficient to characterize species. Coremia formation (Fig. 17) is of no taxonomic significance; however, production of sclerotia is believed to be a constant property, in agreement with Thirumalachar (1955), but not with Gattani (1957), who denied its significance (Figs. 18–20).

Cultural and Biochemical Characteristics

FORMATION OF PIGMENTS

Among the cultural properties of streptomycetes, the color of the substrate growth of the aerial mycelium and the spores and the formation of soluble pigments in organic and synthetic media play a major role in characterizing species. This fact is amply illustrated by the numerous specific epithets referring to color that have been used to designate various organisms. Unfortunately, color characteristics vary greatly with age of the culture, composition of the medium, temperature of incubation, and nature of the inoculum.

Before the introduction of synthetic media, it was a common practice to divide the actinomycetes into two groups: (a) colorless or nonchromogenic, and (b) pigment-producing or chromogenic forms. The latter comprised those organisms that produced deep brown to black diffusible pigments when grown on proteinaceous media. With the introduction of synthetic media, it came to be recognized that different organisms are able to produce a great variety of pigments, ranging from red to blue and from orange and yellow to brown and black. Some are single pigments, and others comprise two or more constituent pigments. Some are water-soluble and others are water-insoluble, as shown in detail in Chapter 13 of Volume I. The presence of oxygen is essential for pigment formation. The pH of the medium greatly affects the nature of the pigments, both insoluble and soluble.

When cultures are grown on optimum sporulation media, the pigmentation of the spores is highly significant; it may be observed at an early growth stage, at maturity, or only in old cultures, since changes in color may occur with age of culture.

The formation of deep brown to black pigments on organic media containing proteins

and protein derivatives, notably the amino acid tyrosine, is an important species characteristic. Certain species may produce only faint brown soluble pigments on organic media, as well as on synthetic media. Different cultures, especially on continued cultivation on artificial media, will show great variation in pigment production.

Since about one third of all species of *Streptomyces* now recognized are melanin-positive (Waksman, 1919; Skinner, 1938), and since this property has been utilized extensively in the classification of actinomycetes, a knowledge of this reaction is of great importance. Gasperini (1891) first utilized this property in dividing the aerobic *Actinomyces* into *A. chromogenus* and *A. albus*. It was later recognized, however, that melanin production is characteristic of a large number of species, including such important forms as the plant pathogen *S. scabies*.

Beijerinck (1900, 1911, 1913) designated as "melanin" the dark pigment produced by *A. chromogenus* from peptone, although this organism did not always produce the pigment from tyrosine. He considered the pigment as a catabolic product of the organic nitrogen.

Lehmann and Sano (1908) first suggested the expression "tyrosinase reaction." They used for their studies a tyrosine-containing medium, melanin being known to be an oxidation product of tyrosine. Waksman (1916, 1919, 1920) expressed considerable doubt that the production of a soluble dark pigment on beef-peptone agar is due solely to this reaction. Gelatin, containing no tyrosine, gives the characteristic pigmentation. Some species producing a typical dark pigment on the beef-peptone agar may fail to do so on synthetic media containing tyrosine.

Skinner (1938) recognized a difference between the dark pigment produced in peptone media and not in tyrosine-containing synthetic media and the black pigment produced in tyrosine (or protein containing tyrosine) media. The tyrosinase reaction was, therefore, considered to be the proper one.

Shinobu (1958) attached great importance to the "tyrosinase reaction" in the species characterization of *Streptomyces*. Ettlinger *et al.* (1958) also recognized the difference between melanin formation and the tyrosinase reaction. In line with the ideas of Beijerinck, Waksman, and Ettlinger *et al.*, recognition will be given here to melanin formation rather than to the tyrosinase reaction.

The formation of yellow, red, blue, green, and other soluble pigments is also highly characteristic of the species growing on synthetic media. There is considerable variation in the intensity of these pigments, depending upon the strain of organism. In view of the fact that color standards are not always available, Lindenbein (1952) suggested a series of color designations which are simple and convenient. This system in a modified form is given in Appendix I.

Pigment formation is considered by Krasilnikov (1960) as a constant specific property, although the nature of the pigments varies with the composition of the medium. The color of the aerial mycelium is not considered as constant and is greatly influenced by the composition of the medium (see also Conn and Conn, 1941).

The variability in pigmentation of different strains of *S. aureofaciens* was studied in detail by Duggar *et al.* (1954) and Backus *et al.* (1954), and is illustrated in Table 7.

UTILIZATION OF CARBON SOURCES

The ability of different species of actinomycetes to utilize as sources of carbon and energy various organic substances, such as carbohydrates, alcohols, salts of organic acids, fats, and amino compounds, can be of considerable diagnostic value. These studies

TABLE 7

Cultural variations of different strains of S. aureofaciens (Duggar*)

Strain No.	Sucrose nitrate agar	Glucose-asparagine agar	Potato	Nutrient agar	Gelatin	Milk
3708	Growth colorless	Growth purplish-brown; aerial mycelium none	Growth dark purplish, lichenoid; color of plug dark, but not black	Growth thin brownish; soluble pigment none	Cream-colored surface ring	Acidified and coagulated, pH 6.03
3709	Growth colorless	Growth cream-colored, aerial mycelium none	Growth light brownish; soluble pigment none	Growth brownish; soluble pigment none	Good liquefaction	?
3710	Growth brownish; aerial mycelium heavy white	Growth cream-colored to faint orange; aerial mycelium white to mouse-gray	Growth light brownish; soluble pigment none	Growth thin brownish; soluble pigment none	No liquefaction?	No visible effect, pH 6.65
3711	Growth colorless	Growth cream colored to faint orange; aerial mycelium white to mouse-gray	Growth orange-colored; soluble pigment none	Growth thin brownish; soluble pigment brownish	Good liquefaction	Coagulation none; peptonization none, pH 7.17
3712	Growth colorless	Growth purplish brown; aerial mycelium none	Growth dark purplish, lichenoid; color of plug dark, but not black	Growth thin brownish; soluble pigment none	No liquefaction?	Surface ring dark brownish; alkaline; coagulation none; peptonization none, pH 7.08
3713	Growth abundant brownish; aerial mycelium abundant white, soluble pigment brownish	Growth cream colored to faint orange; aerial mycelium white to mouse-gray	Growth abundant cream-colored; soluble pigment none	Growth thin cream-colored; soluble pigment none	Good liquefaction	Surface ring; coagulation none; peptonization none, pH 7.17
3714	Growth abundant; aerial mycelium white to faint rose; soluble pigment purplish-brown	Growth orange-colored; aerial mycelium traces of white	Growth abundant brownish; aerial mycelium traces of white	Growth brownish; aerial mycelium white; soluble pigment faint brownish	Good liquefaction	Strong peptonization, pH 7.50
3715	Growth limited	Growth cream-colored; aerial mycelium abundant and gray	Growth orange-colored; soluble pigment none	Growth thin brownish; soluble pigment none	Good liquefaction; brownish pigment	Coagulation and peptonization, pH 6.87

* Personal communication.

date from the early work of Waksman (1919), who employed a synthetic solution to which he added various carbon- or nitrogen-containing compounds. Liquid substrata were employed and cultures were incubated under static conditions. The use of static liquid substrata was later found to give misleading results. In some cases, uniform inoculum distribution is not achieved unless considerable care is taken. Numerous studies (Pridham and Gottlieb, 1948; Benedict *et al.*, 1955; etc.) indicate that solid substrates and different basal media were later used (Table 8).

Hata *et al.* (1953) found a correlation between the groups and types of organisms established on the basis of carbon utilization and their antistreptomytic and antibacterial spectra.

Zähner and Ettlinger (1957) did not attach major significance to the utilization of carbon sources for characterizing species of *Streptomyces*. They suggested that such information be combined with other criteria. None of the 125 cultures they studied could use dulcitol, for example. The best carbon sources for characterizing *Streptomyces* species were found to be raffinose, *l*-xylose, *d*-fructose, *l*-arabinose, and *d*-mannitol. Gordon and Mihm (1959) considered as a species characteristic the utilization of acetate, malate, propionate, pyruvate, and succinate.

None of the actinomycetes produce gas. Some are able to form acid, such as lactic, from certain carbon sources. Gordon and Smith (1954) used acid production from lactose, maltose, xylose, and mannose as one of the criteria for differentiating *Nocardia* and *Streptomyces* species. Gordon and Mihm (1959) later suggested for species separation the use of acid formation from glycerol, glucose, arabinose, erythritol, inositol, lactose, maltose, mannitol, and certain other carbon sources.

TABLE 8

Carbon source utilization by 12 natural variant strains of S. aureofaciens (Backus *et al.*, 1954)

No apparent utilization by any variant	Utilization by all variants	Utilization variable with strain		
			Positive	Negative
Sodium acetate*	Glucose	Galactose	11	1
Sorbose	Sucrose	Sodium citrate*	10	2
Glycine	Maltose			
Mannitol	Starches	Levulose	9	3
Arabinose	Dextrin	Mannose	9	3
	Trehalose	Lactose	9	3
	Glycerol	Magnesium lactate*	7	5
	Sodium succinate*	Xylose	6	6
	Inulin			

* Used at 0.4 per cent level, all others at 1 per cent.

PROTEOLYTIC ACTIVITIES

Among the proteolytic activities of diagnostic value in separating genera, liquefaction of gelatin, hydrolysis of casein, and peptonization of milk are very important.

Species of *Nocardia* effect little, if any, liquefaction of gelatin, whereas most species of *Streptomyces* bring about liquefaction. The rapidity of liquefaction varies greatly. Some species show strong activity, and others give only limited liquefaction. This property, as well as milk peptonization, when combined with the ability of the species to produce brown to black pigments, provides significant criteria for species characterization.

In a study of 477 cultures of *Streptomyces*, Stapp (1953) found only one that did not liquefy gelatin. Detailed studies on proteolytic activities of actinomycetes are found in the work of Waksman (1919), Jensen (1930), Gordon and Smith (1955), and Kutzner (1956), as well as in Vol. 1, pp. 183–186. Waksman (1919) reported that of 35 cultures tested, 33 liquefied gelatin more or less rap-

idly; when these tests were repeated three times, considerable variation in the degree of liquefaction was obtained. Kutzner (1956) kept gelatin cultures for 31 days; only four of 210 failed to liquefy the gelatin. Reports of inability to liquefy gelatin or coagulate or peptonize milk of certain species may often be questioned. Repeated tests with different inocula might have shown different results. Gordon and Smith (1955) suggested casein hydrolysis as one criterion for the separation of *Streptomyces* strains from those of *Nocardia*.

Stapp (1953) reported further that in his collection 18 cultures brought about coagulation of milk without subsequent peptonization, 431 caused coagulation and peptonization, and 19 caused peptonization without previous coagulation. A few cultures occasionally are found that cause no coagulation or peptonization. One wonders whether repeated tests with different inocula might show different results.

REDUCING PROPERTIES

The reduction of nitrate to nitrite has been universally used among the criteria for species differentiation. In view, however, of the influence of nutritional factors upon this reaction, and its quantitative rather than qualitative nature, its significance in species characterization may be questioned.

Proteolysis, starch hydrolysis, sucrose inversion, cellulose utilization, and nitrate reduction were said (Krassilnikov, 1960) to be characteristic of almost all actinomycetes and to have, therefore, no taxonomic significance. Sugar assimilation was considered, however, as a more or less constant property.

UTILIZATION OF NITROGEN SOURCES

As a rule, utilization of nitrogenous compounds has not been considered important for species characterization. Shinobu (1958) considered the utilization of urea, creatinine, and certain amino acids as of some importance in species characterization. Gordon and Mihm (1959) suggested that the ability to attack casein, tyrosine, or xanthine can be considered of some significance in characterizing species.

The use of hydrogen sulfide production as a taxonomic implementation in the differentiation of *Streptomyces* species has been suggested by Pridham (1948). Tresner and Danga (1958) later modified the peptone-iron agar medium. More than 900 strains belonging to one or another of 94 species were tested. There was a marked difference in response from strain to strain within a species; for example, 98 per cent of 227 strains of *S. hygroscopicus* were negative; 99 per cent of 112 isolates of *S. lavendulae* were positive. When employed in conjunction with other physiological, cultural, and morphological criteria, hydrogen sulfide production was said to give promise as an aid in the systematics of the genus *Streptomyces*.

Sensitivity to Antibiotics

Actinomycetes, especially species of *Streptomyces*, have been found in recent years to produce a series of highly valuable chemical substances, notably, antibiotics. This property has come to be considered as highly characteristic of a given species. The fact that a large proportion of all the cultures of *Streptomyces* isolated from natural substrates show some degree of inhibition of growth of other microorganisms, when tested on suitable media, suggested the ability to form antibiotics to be of potential diagnostic value. It is a question whether one is a "lumper" or a "splitter" when one regards the ability to produce an antibiotic as a species rather than a strain characteristic. Certain antagonistic strains belonging to the *S. griseus* group, for example, are able to produce various streptomycins and cycloheximide. Others may form various actinomycins, grisein, streptocin, or candicidin.

It has been suggested that because the

growth of homologous strains of an organism is less inhibited than that of heterologous forms, added weight could be given to the potential diagnostic value of antibiotic production. The application of the concept of antibiosis as a major characteristic for the speciation of actinomycetes is not generally accepted, since the metabolism of these organisms is too complicated to give sharp lines of autoinhibition. At most, it can be a varietal rather than a species characteristic.

Krassilnikov (1950, 1958, 1960a, 1960b) tended to overemphasize the importance of antibiotics in species characterization of actinomycetes. He made two unjustified assumptions: (a) every species synthesizes only one particular antibiotic, (b) antibiotics do not inhibit the growth of organisms belonging to the species producing such antibiotics. Many species and even individual cultures are able to form a variety of different antibiotics; on the other hand, the same antibiotic may be produced by different organisms. The growth of an organism may in some instances actually be inhibited by its own antibiotic, as with *S. fradiae* and neomycin. Finally, a single culture may produce mutants which either have lost the ability to form a particular antibiotic or have gained the ability to synthesize a totally different antibiotic. It is somewhat dangerous to use assumptions and generalizations as the basis for species characterization. The importance of considering antibiotic formation in the systematization of actinomycetes has also been emphasized by Gause (1955).

Actinophage Sensitivity

During the last 10 years several attempts have been made to determine whether "phage-typing" of actinomycetes might be of some help in identifying unknown isolates. The results obtained point to several facts which must be kept in mind if one tries to use this procedure for characterizing and classifying *Streptomyces* species.

1. Actinophages vary greatly in their host ranges.

a. Most actinophages which were tested against a large number of organisms proved to be polyvalent; that is, they lyse cultures that belong to different species or even genera (different according to our present species concept, which is based on the system presented here). The data presented by Bradley and Anderson (1958) might serve as an illustration (Table 45, Volume I). The activity of some phages upon members of the genera *Streptomyces* and *Nocardia* led these workers to question the validity of separating these two genera, which have actually been placed in two different families within the order *Actinomycetales*. Activity of a polyvalent *Streptomyces* phage on two *Nocardia* species was also found by Gilmour *et al.* (1959). In a study carried out by St. Clair and McCoy (1959), however, nine phages which proved to be polyvalent against several *Streptomyces* species failed to attack any of the four *Nocardia* species tested. The polyvalent character of other phages tested against other species has been shown also by other investigators (Hoehn, 1949; Chang, 1953, Rautenstein and Kofanova, 1957; Gause *et al.*, 1957; Mach, 1958; Shirling, 1959a, b; Kutzner and Waksman, 1959a; Kutzner, 1960). Obviously, therefore, a phage characterized by a wide host range is usually of little value in species differentiation, unless one is inclined to be a "lumper" who demonstrates by the use of a polyvalent phage that he is right and the "splitters" are wrong.

b. Some phages have been found to be specific, causing the lysis of strains of only a few species or of only certain strains of one species. In the latter case one might be justified in doubting the uniformity of the species rather than in considering the phage as "superspecific." This seems to be definitely true of the species "*S. griseus*," various

strains of which show a very different sensitivity pattern against certain phages.

It is true, likewise, of the separation of streptomycin-producing strains from griseinproducers and other members of the former *S. griseus*, which is now regarded as a species group rather than a single species (Waksman, 1959). There have been various reports concerning phages which are active upon streptomycin-producing strains, but do not attack grisein-producers or nonantibiotic-forming cultures (Woodruff *et al.*, 1947; Waksman *et al.*, 1947; Reilly *et al.*, 1947; Waksman and Harris, 1949; Hoehn, 1949; Carvajal, 1953; Burkholder *et al.*, 1954). Some streptomycin-producing cultures have been found, however, that are resistant to these specific phages (Okami, 1950; Carvajal, 1953; Kutzner, 1960). Other phages have been discovered that are specific against *S. coelicolor* (Kutzner and Waksman, 1959a; Kutzner, 1960), *S. lavendulae* (Gause *et al.*, 1957; Shirling, 1959), and *S. olivaceus* (Khavina and Rautenstein, 1958).

2. No general conclusion can be drawn from the spectrum of a polyvalent phage in regard to relationships between lysed strains. However, a polyvalent phage can be useful in taxonomic studies if it shows specificity within a particular group of organisms that are very similar in their other properties and therefore hardly distinguishable. Further, testing several polyvalent phages might result in typical sensitivity patterns of the organisms which might be of some value in recognizing whether one has to do with closely related or unrelated organisms.

3. Actinophages vary greatly when tested against numerous strains. In some cases differences in plaque counts might be due to host range mutants which are present at a concentration of 10^3 to 10^5 particles. These mutants would attack a "new host" resistant to the parent phage, as shown by Welsch (1954, 1957) and Welsch *et al.* (1957). In numerous other cases, however, the development of host range mutants cannot explain the wide host range, and the phages retain their polyvalent nature even after several serial passages on heterologous hosts (Chang, 1953; Shirling, 1959a; Bradley, 1959; Gilmour *et al.*, 1959). It is necessary to carry out phage tests with different dilutions of the original phage preparation, which should contain about 10^7 to 10^9 particles per milliliter.

4. A survey of the literature shows that almost every investigator uses a different medium for phage typing. The methods comprise either spot tests or single plaque counts. In a comparative study of different media for phage typing, Kutzner (1960) found that some phages gave similar plaque counts on a variety of media. However, counts of phages that formed tiny plaques were found to be quite dependent on media composition. Inorganic salt content of media was found to influence plaque counts most strikingly. Some phages gave no plaques on media containing NaCl but gave high plaque counts when plated on the same medium without NaCl (with or without $CaCl_2$), while other phages showed higher activity on NaCl than on $CaCl_2$ media. The expression of phage activity is apparently influenced by a great many unknown factors. One of the phages lysed some strains with a medium containing NaCl and gave high plaque counts, but showed no activity against other strains on the same medium. These results suggest that a medium found optimal for one host-phage system might be quite unsuitable for another. Before a phage is typed against a large number of strains, an optimal medium must be developed. Better still, tests should be carried out with several different media, selected for their usefulness with particular strains.

Serological Reactions

Use of serological techniques, particularly those of agglutination and precipitation, has

been suggested for species identification of actinomycetes. Aoki (1935–1936) was thus able to differentiate between representatives of three genera, *Actinomyces*, *Nocardia*, and *Streptomyces*. By means of sonic vibrations, Ludwig and Hutchinson (1949) prepared antigen suspensions satisfactory for use in agglutinin and precipitin reactions and for the production of immune sera in rabbits. Use of such suspensions in the identification of actinomycetes was suggested by Yokoyama and Hata (1953) and Hata *et al.* (1953). A purified antigen of a streptomycin-producing strain was found active against immune sera of the same strain, but not against sera of other antibiotic-producing organisms. These investigators were thus able to establish the close relationship of luteomycin- and chloramphenicol-producing organisms.

Ochoa and Hoyos (1953) found a correlation between microscopic morphology and serological reactions which made it possible to divide the actinomycetes into four groups: Group 1, including species of *Actinomyces* and *Nocardia*; Group 2, made up largely of *Nocardia*; Groups 3 and 4, comprising species of *Streptomyces*. Slack *et al.* (1951), however, found that antisera prepared with *A. bovis* brought about low titer agglutination of *Nocardia* and of two species of *Streptomyces*. They concluded that a close antigenic relationship exists between members of the genus *Actinomyces* and that there is a group relationship among *Actinomyces*, *Nocardia*, and *Streptomyces*. Okami (1956) found definite antigenic relationships between strains of closely related forms of *S. lavendulae*. See also Tanaka *et al.*, 1959.

Chemical Composition

A detailed study of the chemical composition of cells of actinomycetes has been presented in Volume I (pp. 158–163).

The occurrence of specific chemical compounds in the cells of the organisms suggests possible differentiation between groups of actinomycetes. This is true, for example, of the occurrence of diaminopimelic acid, a constituent that may prove to be of generic rather than specific significance. Romano and Sohler (1956) and Sohler *et al.* (1958) have shown that cell walls of streptomycetes can be solubilized by lysozyme, suggesting the presence of a mucopolysaccharide; on the other hand, cell walls of nocardiae do not possess this property.

Ecology

The natural substrate of an organism, especially diseased plants or animals, and composts of stable manures and plant residues at high temperatures, is of some systematic significance. Various attempts have been made to utilize the ecological characteristics of the actinomycetes as a basis of classification. Thus, the following rather broadly defined ecological categories have been proposed at various times to classify actinomycetes:

a. Animal parasites.
b. Plant parasites.
c. Soil inhabitants.
d. Water inhabitants.
e. Mesophilic forms.
f. Thermophilic forms.
g. Inhabitants of acidic (pH 3 to 6.5) substrates.
h. Inhabitants of neutral to alkaline substrates (pH 6.5 and above).

The temperature at which an organism is grown greatly affects the nature and amount of growth, the nature and extent of sporulation, and the degree of formation of soluble pigments. The optimum temperature for the growth of most species of *Streptomyces* is between 25 and 30°C. Only a few of these organisms are thermophilic. Abilities to grow under mesophilic and thermophilic conditions have been recognized as important criteria for establishing species and even genera of actinomycetes and other microorganisms.

The optimum reaction for the growth of actinomycetes is pH 6.8 to 7.5. When these organisms are grown on complex organic media, and on many synthetic media, the reaction usually becomes alkaline. Some actinomycetes, however, are able to grow at pH 4.5 to 6.5 and even at pH 3.0 to 4.5. Such forms are not common, but the reaction of the substrate has been recognized as a potential diagnostic property.

On the basis of their effects on dead residues and upon living forms of life, actinomycetes have been grouped as saprophytes and parasites, the latter being further grouped into plant and animal parasites. Thus we speak of "actinomycosis," caused by *A. bovis* and *A. israelii*, and "nocardiosis," caused by different species of *Nocardia*. We associate *S. scabies* with the "scab" of potato tubers, and *S. ipomocae* with a disease of sweet potato roots.

Genetics

Little is known about the genetic properties of actinomycetes and their possible bearing upon problems of classification. Certain observations have been made recently, however, which offer rather promising leads in establishing species relationships. The concept of vegetative hybridization of *Streptomyces* cultures has been suggested. By repeated growth of a culture in a sterile filtrate of sand-macerated mycelium of another culture, the former undergoes morphological and physiological changes. The significance of this phenomenon and its potential utilization for species characterization are still to be elucidated. Sermonti and Spada-Sermonti (1956) demonstrated several types of recombination among "wild" and mutant strains of *S. coelicolor* (most probably *S. violaceoruber*). It has been brought out in Chapter 6 of Volume I that true hybrids can be obtained by mating two different mutant strains of an actinomycete. Welsch (1958) suggested that mating may offer a convenient criterion for the practical definition of a species. The assumption was thereby made that a species is distinct if it does not cross, or gives only unfertile crosses with other similar species.

Type Cultures

An important, and often-used technique in species characterization of actinomycetes is that of comparing fresh isolates with type cultures. For the higher forms of plant life, species characterization is facilitated by study of preserved herbarium specimens. For microorganisms, special collections of named cultures are available for study. These cultures allow comparisons of living material, since dead or dried cultures are of but little significance.

In establishing type cultures of actinomycetes it is important to keep in mind the fact that such cultures undergo considerable variation when grown for a long time upon artificial media. Some of the cultures may lose their ability to produce aerial mycelium and are thus deprived of properties of major diagnostic value. Unknown strains of *Streptomyces* free from aerial mycelium may even be considered as species of *Nocardia*.

According to Pridham,* if reasonably fresh isolates were maintained on the proper media and preserved by lyophilization, the individual laboratory would experience far fewer difficulties than have been experienced in the past. Pridham reported that since 1953, with the use of these media and techniques, very rarely have strains been found that produce no aerial mycelium (which is generally well sporulated) on the isolation media; a very low incidence of strain degeneration has been noted in active cultures as determined by the methods of assessment, and all isolates have been routinely lyophilized. These lyophil tubes, opened from time to time, have been found to give cultures that are

* Personal communication.

FIGURE 21. Formation of straight sporophores by *Streptomyces* sp., × 1500 (Courtesy of Miss A. Dietz, Dept. of Microbiology, Upjohn Co., Kalamazoo, Mich.).

equivalent to the original soil isolates, as determined by the methods of assessment used.

The prior growth of the organism in soil media (sterile soil treated with a small amount of $CaCO_3$, if acid, and with a half per cent of dried blood) or in carbon- or nitrogen-poor media, its refrigeration or its lyophilization—each or all tend to prevent degeneration and thus preserve the original characteristics of the type culture. The cultures that have already degenerated will tend to regain their original properties as a result of such treatments.

Shinobu (1958) suggested the following method for making a soil medium: Into a test tube of 1.5 cm diameter place 7 g dried fertile soil; add 1.5 ml of 2 per cent solution

of glycerol; make up the water content to about 20 to 25 per cent. Sterilize the tubes at 20 pounds for 20 minutes. The culture strains are inoculated on this medium and incubated at 28–30°C. When the growth of the organism is successful, white aerial mycelium appears first on the surface of the soil; when the culture matures the characteristic color of the aerial mycelium is produced.

There is always the danger that an old culture, transmitted from one laboratory to another, may either have become modified or have lost some of its original properties. It may have become contaminated, and the contaminant may eventually replace the original culture. One must also remember that different investigators have often based their descriptions of a particular species not upon the original culture but upon subsequent isolates, which may or may not represent the same species.

Finally, many holo-type cultures are not available at all. Therefore, in some cases type cultures are not reported. When reported, they usually refer to the Institute of Microbiology Collection (IMRU), the American Type Culture Collection (ATCC), or to the Agricultural Research Service Culture Collection of the Northern Regional Research Laboratory, U. S. Department of Agriculture (NRRL). Other collections include Eidige Technischen Hochschule (ETH) Zürich; Centraalbureau voor Schimmelcultures (CBS) Baarn; and Institute of Applied Microbiology, University of Tokyo (IAM).

Standard Media

Some media are more favorable than others for sporulation of *Streptomyces* cultures. In view of the importance of sporulation in characterizing a species (also in placing an organism in the proper genus), it is essential to select favorable media. Furthermore, since some forms tend to lose the property of sporulation on continued growth, special precautions must be taken in preserving such cultures. The loss of aerial mycelium may be reversible or irreversible. Since nonsporulating streptomycetes may resemble nocardiae and since certain nocardiae have been reported to produce aerial mycelium and spores similar to those of typical *Streptomyces* cultures, the element of confusion between the two genera always exists.

Description of Streptomyces Species

It is commonly believed that to characterize a species it is essential to describe a large number of its morphological and physiological properties. This procedure is not always helpful, especially if based upon unreliable criteria. The medium may not be readily duplicated, or conditions of growth may be different, or the inoculum may not be prepared in the same way. Because of these and other variations, many cultures recently isolated have been described as new species. Another reason is that it is much easier to create a new species than to attempt to correlate the characteristics of a freshly isolated culture with those of known species already described in the literature. Numerous new species also have been created to facilitate the obtaining of patents.

Hesseltine *et al.* (1954) suggested that the following steps be taken in the taxonomic study of a *Streptomyces* species:

1. Collection of strains on the basis of pigmentation of aerial mycelium.

2. Study of the morphology of strains growing on a number of media favorable to sporulation.

3. Examination of the color of spores of strains growing on optimum sporulating media. Five color groups were recognized: (a) lavender, red, or pink; (b) blue, blue-green, or green; (c) yellow; (d) white; (e) gray, gray-brown, olive-gray, or dark gray.

4. Study of cultural characters of strains on various synthetic and organic media.

5. Analysis of certain physiological and biochemical properties, notably action on gelatin, starch, milk, and peptone-iron agar; nitrate reduction; utilization of carbon and nitrogen compounds; antibiotic action, comprising formation of and sensitivity to antibiotics.

6. Identification of new strains with known species, and preservation of cultures.

Pridham* emphasized that recently he has been placing principal emphasis on micromorphology, secondary emphasis on chromogenicity (deep brown to black diffusible pigments), and tertiary emphasis on color of aerial mycelium.

In an effort to determine whether freshly isolated cultures can be identified on the basis of published descriptions and what conditions justify the creation of new species, several obvious comparisons were made (Waksman, 1957). Certain strains that might be included in various important species or group-species were critically examined. The following conclusions were reached:

At present, various morphological, cultural, and biochemical properties are known which make it possible to establish definitely certain distinct species among the actinomycetes. Some of these characters are constant within certain conditions of nutrition and environment, others are variable. Certain additional properties may be required in order to establish the degree of variation of a culture before it can be recognized as a new species.

Certain categories of relationships among the actinomycetes must be taken into consideration in order to establish definitely the systematic position of a given culture. These may be briefly summarized as follows:

1. On the basis of all the accumulated evidence, actinomycetes are shown to belong definitely to the bacteria.

2. The position of the true actinomycetes in relation to related bacterial forms, notably the mycobacteria and corynebacteria, must be recognized; this is true especially of certain nocardial types.

3. The generic interrelationships among the actinomycetes are highly significant. The separation of members of the genus *Streptomyces* from those of *Nocardia* is difficult, especially when one is dealing with nonsporulating forms of the first and sporulating forms of the second. The recent addition of two new genera, *Actinoplanes* and *Streptosporangium*, and the recognition of certain thermophilic groups as separate genera add further problems to these generic interrelationships.

4. Within each genus, certain groups, species-groups, or series must be recognized. A combination of morphological and cultural properties permits the establishment of species-groups. Some of these comprise a large number of forms with many variable characteristics.

5. Differentiation of individual species within each group is based upon a combination of cultural and biochemical properties. The production of specific antibiotics and the utilization of different sugars are ample illustrations of this.

6. Cognizance of the strains and varieties within each species must finally be taken. This may be based upon certain qualitative properties, such as sensitivity to phages, or quantitative properties, such as production of a given antibiotic, vitamin, or enzyme, or sensitivity to a given antibiotic.

The fact that a culture becomes important for the production of a particular metabolic product, such as an antibiotic, an enzyme, or a vitamin, may impart to the culture particular significance for characterization purposes.

The existence of physiologic races or varieties among species of actinomycetes, especially among those placed in the genus *Streptomyces*, has been fully recognized. Just as in improving higher forms of life one is always faced with the selection of varieties resistant to disease, or giving higher yields, or having other desirable qualities, so one must select strains of actinomycetes on the basis of resistance to phage or of production of higher yields of a given antibiotic or other metabolic product.

* Personal communication.

Chapter 5

Systems of Classification and Identification of Groups and Species of the Genus *Streptomyces*

Principles of Separation of Genera

The historical background and various systems of classification of the order *Actinomycetales* in general and of the actinomycetes in particular have been discussed in detail in Chapter 4 of Volume I. The principles underlying the generic and specific separation of the organisms are presented in Chapter 4 of the present volume. The variability and overlapping among genera and species have been emphasized in Chapter 6 of Volume I. Certain important factors pertaining specifically to the genus *Streptomyces* must be considered before any discussion is presented of the separation of this genus into subgenera, series (species-groups), species, and varieties.

Among the factors that must be emphasized in any attempt to classify actinomycetes, the following three are most important: (a) the nature of the substrate (or vegetative) growth and the nature of the aerial mycelium, if any; (b) the degree of variability of the cultures; and (c) the effect of the composition of the medium. To facilitate recognition of the organisms and to establish constant and variable differences for classification purposes, well-defined media and standard conditions of cultivation must be used.

Actinomycetes are differentiated from the true bacteria by their filamentous growth and by their true branching. It is often difficult, if not impossible, to distinguish between the profuse branching of certain mycobacteria and the short-lived mycelium of the nocardias, except for the fact that the latter produce mycelium consistently in the early stages of their development. There is a gradual transition between the mycobacteria and the nocardias. It also is often difficult to differentiate between the nocardias and the streptomyces. The latter are characterized by the constant and marked nature of their aerial mycelium, whereas the nocardias are characterized largely by the transitory and undifferentiated nature of this mycelium.

In establishing differences between nocardias and streptomyces, one must consider the following factors:

1. Nocardias usually have been considered incapable of forming aerial mycelium that could be differentiated from the substrate mycelium. It also has usually been assumed that no spirals are ever formed from the mycelium. Recently, however, Gordon and Mihm (1958) have reported that certain nocardias are able to form aerial mycelium similar to that of streptomyces

and that spirals also may be formed. A streptomycete forms a characteristic aerial mycelium. This property may be lost, however, on continued cultivation or under special conditions of treatment. The aerial mycelium frequently develops characteristic spirals, tufts (Fig. 22), or verticils (whorls).

2. A streptomycete usually multiplies by the concentration and fragmentation of the protoplasm within a filamentous cell, followed by the dissolution of the cell membrane. The fragmented portions of the mycelium usually develop, under favorable conditions, into fresh mycelium, either by germ tubes or by lateral budding. Spores or conidia are produced. The substrate mycelium does not segment spontaneously into bacillary or coccoid forms, but remains nonseptate and coherent even in old cultures, thus forming the characteristic tough textured, leathery growth.

3. In nocardias, the aerial hyphae are believed to represent an upward extension of the substrate mycelium, and usually do not exhibit any differentiated protoplasm. When a streptomycete loses its capacity to produce aerial hyphae, a form analogous to that of a nocardia may result, except for the structure of the mycelium and that faculty of the degenerated culture to regain the lost capacity.

4. Another difference between nocardias and streptomycetes is the acid-fastness or partial acid-fastness of some of the former when grown in certain media; the latter are never acid-fast.

As pointed out in Chapter 1, Gordon and Smith (1955) proposed six distinctive characters for the separation of the two genera. These criteria are: (a) colony structure; (b) casein hydrolysis; (c) dissolution of tyrosine and xanthine; (d) acid production from glucose and glycerol; (e) lack of acid formation from arabinose, xylose, lactose, mannitol, and inositol; and (f) utilization of acetate, propionate, pyruvate, malate, and succinate.

Strains of organisms giving positive results in five or six of the physiological tests belong to *Streptomyces*; strains with four to six negative reactions, to *Nocardia*.

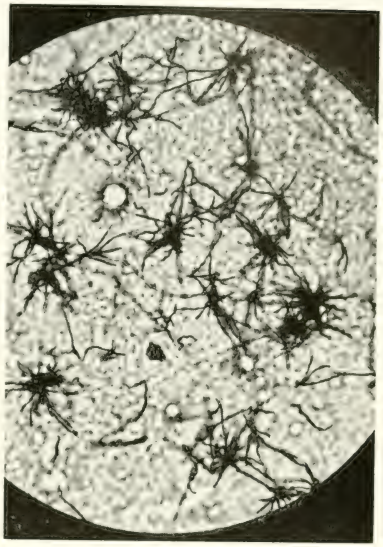

FIGURE 22. Tuft formation in aerial mycelium of the *Streptomyces griseus* type.

Although considered as somewhat arbitrary, these criteria allowed clear-cut generic separation of 97 per cent of 251 strains studied, regardless of their morphological variation. Ninety-six per cent of the strains received as *Streptomyces* were positive in five or six of the following reactions: hydrolysis of casein, dissolution of tyrosine, and acid production from xylose, mannose, maltose, and lactose. Strains that no longer formed aerial hyphae and spores, but known to be descendants of typical sporulating ones, also were positive in five or six of these tests. Two-thirds of the strains labeled *Nocardia* gave negative results in four to six of the same tests. Of the remaining third of the strains received as *Nocardia*, 24 had the same reactions as accepted strains of *Streptomyces* and were assumed to

be mislabeled; seven were listed temporarily as intermediates between the two genera.

The differentiation between *Streptomyces* and the other genera of actinomycetes is not very difficult. The formation of aerial mycelium and the manner of sporulation are markedly distinct for *Streptomyces* as compared to *Micromonospora*. Species of *Thermoactinomyces* also produce an aerial mycelium, similar to that of species of *Streptomyces*, but they form single spores, similar to those of *Micromonospora*. The other thermophilic genera, as well as the genera *Waksmania* (*Microbispora**), *Actinoplanes*, and *Streptosporangium* also can be differentiated from *Streptomyces*, as shown in Chapters 8 to 11.

Among the numerous species belonging to the various genera of actinomycetes, those of the genus *Streptomyces* are by far the most important, largely because of their wide distribution, their greater abundance, and their ability to produce antibiotics and vitamins and to carry out important chemical conversions. Hence a detailed consideration of this genus is justified.

Description of Genus *Streptomyces*

Streptomyces species produce a well-developed mycelium. The diameter of the hyphae seldom exceeds 1.0 μ and is usually only 0.7 to 0.8 μ. The hyphae vary greatly in length: some are long with limited branching; others are short and much branched. The substrate mycelium does not form cross walls; it does not break up into rod-shaped and coccus-like bodies. Reproduction occurs by means of spores or by bits of mycelium. Spores or conidia are formed in special spore-bearing hyphae or sporophores which arise from the aerial mycelium either monopodially or in the form of tufts or verticils. The sporulating hyphae are straight or curved. The curvatures range from mere waviness to perfect spirals, which may be compact, in the form of fists, or long and open (Fig. 23).

The spores of streptomycetes comprise four types: smooth, warty, spiny, or hairy. About one-third of the gray- to brownish-spored species were found (Tresner *et al.*, 1960) to form spiny, warty, or hairy spores; the remainder were smooth-spored. All the blue- to blue-green-spored forms had spiny spores. White, yellow, cream, or buff types had smooth-walled spores. All the pinkish-tan-spored group had smooth spores, with the exception of *S. erythreus* and *S. purpurascens* which had spiny spores. The conclusion was also reached that, because of the variation of spore size and shape, those properties are of limited usefulness for taxonomic differentiation.

The growth of *Streptomyces* "colonies" on artificial media is smooth or lichenoid, hard and densely textured, raised, and adhering to the medium. The colony is usually covered completely or partially (in the form of spots or concentric rings) by aerial mycelium, which may be variously pigmented, depending on the species and on the composition of medium. In liquid media, especially in shaken cultures, growth of streptomyces is usually in the form of flakes, which gradually fill the container, or in the form of spherical growths; the former type of growth is the more desirable from the point of view of antibiotic production.

Many of the cultures, either in the form of colonies on the surface of solid media or as flaky growth in submerged culture, may undergo rapid lysis. The production of antibiotics usually corresponds with the lysis of the cultures. Frequently, the lysis is brought about by a phage, known as actinophage, which exerts an injurious or destructive effect upon the mycelium.

* Both designations were published, in different journals, the same month and the same year. Priority has not been definitely established.

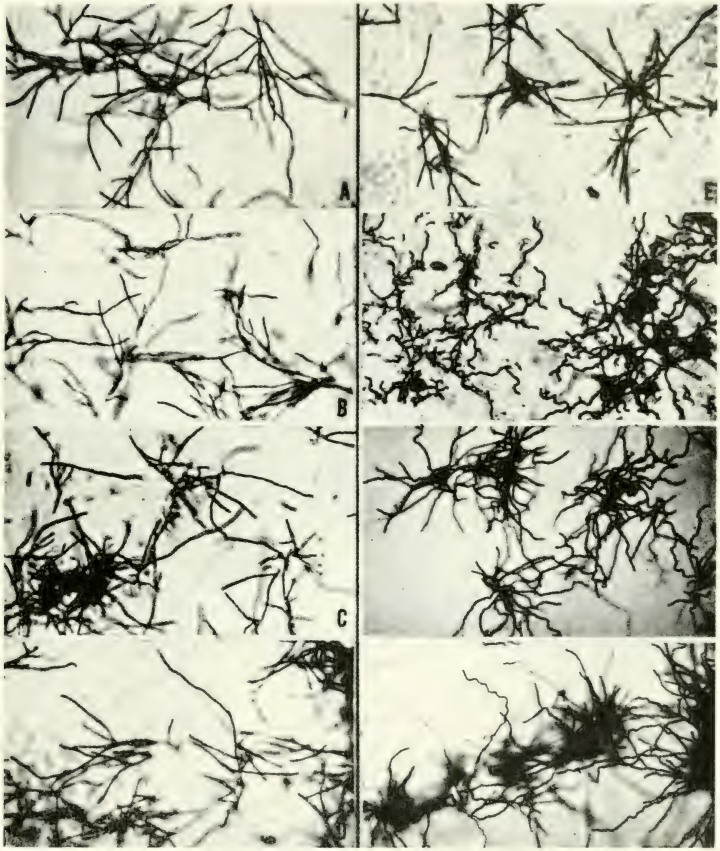

FIGURE 23. Various strains of *Streptomyces californicus* (Reproduced from: Burkholder, P. R. *et al.* Bull. Torrey Botan. Club **82**: 111, 1955).

Classification Systems of the Genus *Streptomyces*

Early Systems of Classification

Although the earlier systems of classification of actinomycetes were also supposed to be concerned with all the forms usually included in this group, they represented largely those forms that are now included in the genus *Streptomyces*. This is true, for example, of the first classification of Sanfelice (1896), and the subsequent ones of Krainsky (1914) and of Waksman and Curtis (1916). Only the more comprehensive and more recent systems are presented here. The earlier ones were given in Chapter 4 of Volume I.

1. WAKSMAN AND CURTIS (1916) SYSTEM

This system was based upon formation of soluble pigments in organic media, rate of gelatin liquefaction, and structure of aerial mycelium.

A. Gelatin rapidly liquefied; no brown pigment.
   1. Spirals formed.
      1. No soluble pigment on synthetic media.
         Actinomyces Rutgersensis
      2. Pigment formed on synthetic media.
         a. Pigment dark blue.
            Actinomyces violaceus-Caeseri
         b. Pigment brown.
            Actinomyces diastaticus
   II. No spirals.
      1. No soluble pigment.
         a. Growth orange-red, aerial mycelium white.
            Actinomyces albosporeus

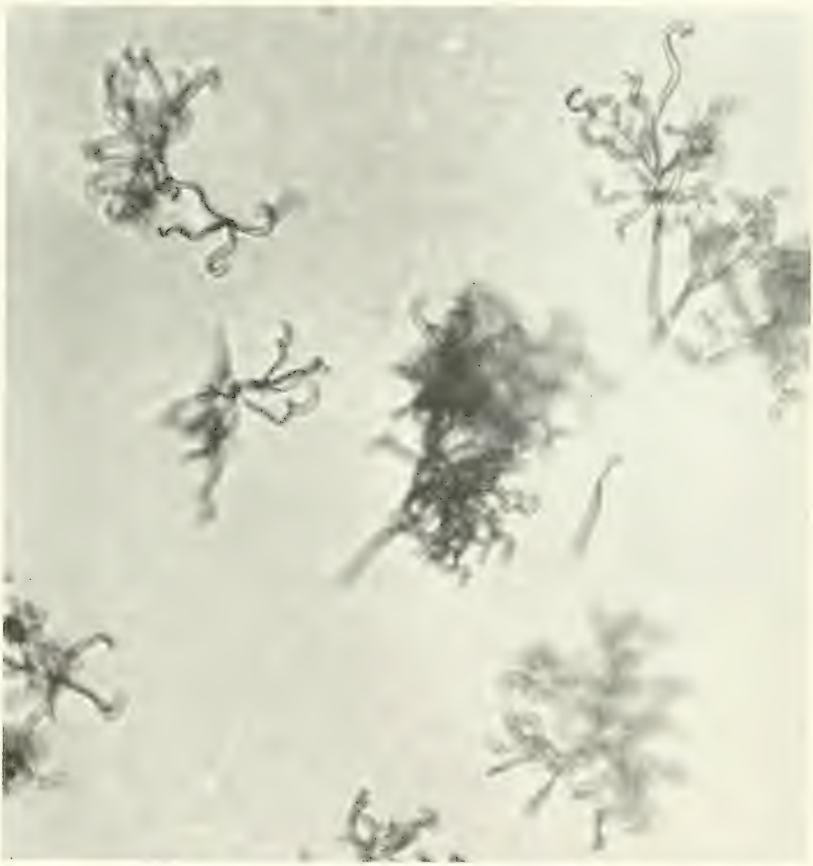

FIGURE 24. Sporophores of *Streptomyces* sp. producing spirals, × 1500 (Courtesy of Miss A. Dietz, Dept. of Microbiology, Upjohn Co., Kalamazoo, Mich.).

b. Growth rose-colored, aerial mycelium rosy.
Actinomyces Fradii
c. Growth a mixture of white and yellow.
a¹. No conidia.
Actinomyces albo-flavus
b¹. Abundant conidia.
a². Conidia rod-shaped, powdery, gray-yellow.
Actinomyces griseus
b². Conidia spherical and oval, growth compact, citron-yellow.
Actinomyces citreus
d. Growth at first colorless, then brown to black.
a¹. Aerial mycelium white.
Actinomyces alboatrus
b¹. Aerial mycelium dark gray.
Actinomyces Lipmanii
2. Soluble pigment produced.
a. Soluble pigment green.
Actinomyces Verne
b. Soluble pigment dark blue.
Actinomyces violaceus-niger

B. Gelatin rapidly liquefied; brown pigment formed.
I. Spirals produced.
a. Growth rose-colored; aerial mycelium rosy.
Actinomyces roseus
b. Growth colorless; aerial mycelium golden brown.
Actinomyces aureus
c. Growth slightly brown; aerial mycelium white.
Actinomyces Halstedii
II. No spirals.
1. No soluble pigment on synthetic media.
a. Growth red to red-orange; no aerial mycelium.
Actinomyces Bobili
b. Growth white; aerial mycelium white.
a¹. Aerial mycelium thin, rare, netlike.
Actinomyces reticuli
b¹. Aerial mycelium thick, white to gray.
Actinomyces albus
2. Soluble brown pigment produced on synthetic media.
a. Aerial mycelium white, abundant.
Actinomyces diastato-chromogenus
b. Aerial mycelium white, produced late or not at all.
Actinomyces chromogenus group
c. Growth green; aerial mycelium white.
Actinomyces virido-chromogenus

C. Gelatin slowly liquefied; no soluble pigment.
I. Spirals produced in aerial mycelium.
1. Soluble red and blue pigments.
Actinomyces violaceus-ruber
2. No soluble pigment; substrate growth red.
Actinomyces Californicus
II. No spirals produced in aerial mycelium.
1. Growth yellow; no soluble pigment.
Actinomyces parvus
2. Growth tends to crack; soluble brown pigment.
Actinomyces exfoliatus

D. Gelatin slowly liquefied; brown pigment produced.
I. Spirals produced; aerial mycelium lavender.
Actinomyces lavendulae
II. No spirals.
1. Growth yellow; aerial mycelium gray.
Actinomyces flavus
2. Growth colorless; aerial mycelium purplish-white.
Actinomyces purpurogenus
3. Growth black; aerial mycelium scant.
Actinomyces erithrochromogenus
4. Growth purple; no aerial mycelium.
Actinomyces purpeo-chromogenus

2. WAKSMAN SYSTEM (1919)

This was a modification of the previous system and was based upon a study of 41 species. An examination was made of the morphology of the aerial mycelium on two media; growth, aerial mycelium, and soluble pigment on 12 different media; various biochemical properties, such as carbon and nitrogen utilization, proteolytic activities, diastase and invertase formation, reduction of nitrate to nitrite, and change in reaction of medium. A brief outline is presented here:

A. Soluble pigment produced on organic media.

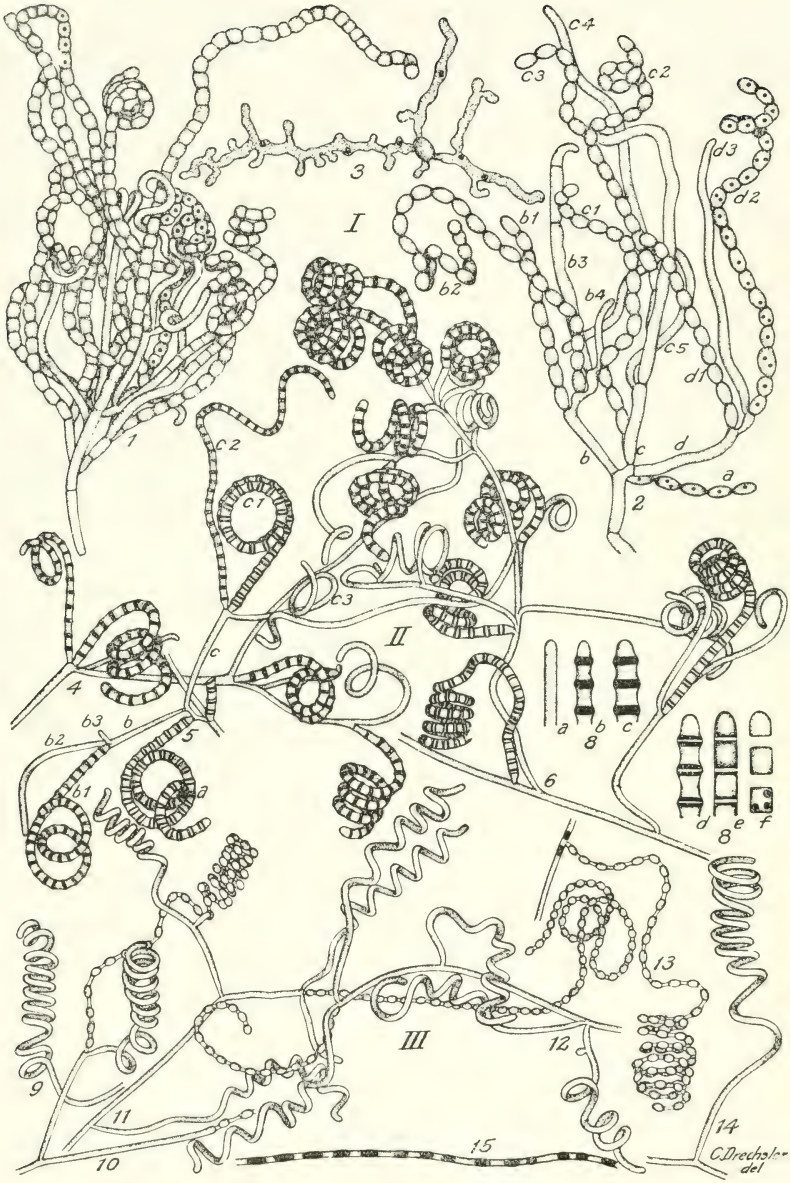

FIGURE 25. One of the early studies on the structure of the aerial mycelium and the manner of sporulation of *Streptomyces* cultures (Reproduced from: Drechsler, C. Botan. Gaz. **67**: 66–83, 147–168, 1919).

I. Pigment deep brown.
II. Pigment faint brown, golden yellow, or blue.

B. No soluble pigment on organic media.
I. Strongly proteolytic.
II. Weakly proteolytic.

The need for the recognition of species-groups was also emphasized: "All the cultures should be divided into groups, the representatives of which have common morphological, physiological and cultural characters. These species-groups may show slight variations within the groups, when several representatives are compared, but all of them possess in common the main distinguishing characters of the species, and are distinctly different from any other species-group."

3. JENSEN'S SYSTEM (1930)

Jensen modified the above system of Waksman, in describing 90 strains of actinomycetes, largely streptomycetes, isolated by him. These strains were divided into several species-groups.

A. No pigment produced on protein media.
1. Red or blue pigments in synthetic media; marked reduction of nitrates.
Actinomyces violaceus-ruber
2. No red or blue pigments.
a. Typical golden pigment in all synthetic media.
Actinomyces fulvissimus
b. Pigment not typical; abundant aerial mycelium.
a¹. Aerial mycelium on synthetic media dark slate-gray; lemon- or sulfur-yellow pigments sometimes formed.
a². Vegetative mycelium on synthetic agar light colored.
Actinomyces cellulosae
b². Vegetative mycelium on synthetic agar turning dark.
Actinomyces olivaceus
b¹. Aerial mycelium greenish- or yellowish-gray; very rapid liquefaction of gelatin and blood-serum.
a². Aerial mycelium greenish.
Actinomyces griseus
b². Aerial mycelium yellowish.
Actinomyces griseoflavus

B. Typical brown pigment in protein media ("chromogenus" species).
1. Deep brown growth and pigment in all synthetic media.
Actinomyces phaeochromogenus
2. Pigment in synthetic media of other color or absent.
a. Aerial mycelium absent or in traces; typical red vegetative mycelium.
Actinomyces bobili
b. Aerial mycelium more or less abundant.
a¹. Typical red pigment in synthetic agar.
Actinomyces erythrochromogenus
b¹. Pigment not red.
a². Aerial mycelium rose to cinnamon-brown.
Actinomyces roseus
b². Aerial mycelium abundant, characteristic lead-gray; light brown pigment in synthetic media.
Actinomyces diastatochromogenus

In a subsequent contribution, Jensen (1931) emphasized again that the term "species" as applied to actinomycetes should be used in the sense of Waksman's "species-groups," or a "broad group of strains agreeing in certain outstanding morphological and biological features"; otherwise, "every strain of actinomycetes isolated from a plating from an ordinary soil could then be raised to the rank of species."

4. KRASSILNIKOV SYSTEM (1941)

This system was based primarily upon the morphology of sporophores of the cultures and the shape of their spores, and secondarily upon the pigmentation of the cultures.

A. Sporophores branching monopodially.
I. Spiral-shaped sporophores, produced on hyphae of aerial mycelium.
1. Spores spherical or oval.
a. Cultures colorless, not producing any pigmentation.

a¹. Aerial mycelium white.
a². Saprophytes, living on dead material.
Actinomyces albus
b². Parasites, living on plants.
Actinomyces totschidlowskii
b¹. Aerial mycelium dark gray.
Actinomyces griseus
c¹. Aerial mycelium green.
Actinomyces glaucus

b. Cultures pigmented blue.
a¹. Pigment of the anthocyanin type, similar to litmus.
Actinomyces coelicolor
b¹. Blue pigment not changing with acidity of medium.
Actinomyces cyaneus

c. Cultures violet, forming two basic pigments (red and blue), both dissolved into the substrate.
a¹. Cultures not forming any fluorescent substance in liquid media.
Actinomyces violaceus
b¹. Cultures producing in synthetic media a fluorescent substance of blue-green color similar to pyocyanin.
Actinomyces pluricolor

d. Cultures black-violet, forming red and blue pigments, as well as a brown pigment of the type of melanin, which changes the violet color of the culture to violet-black.
Actinomyces violaceus-niger

e. Cultures red-colored, producing pigments insoluble in water, of the lipoactinochrome type; color of medium not changing with acidity.
a¹. Cultures not forming any brown or black pigments; they are always red, sometimes with a brownish tinge, but not black.
Actinomyces ruber
b¹. Cultures producing on synthetic media, in addition to pigments, a black or dark brown substance which gives the culture a red-brown to black color.
Actinomyces melanocyclus

f. Cultures yellow, citron-yellow, or brownish-yellow.
a¹. Saprophytes.
Actinomyces flavus
b¹. Living on plants.
Actinomyces setonii

g. Cultures orange.
a¹. Saprophytes.
Actinomyces aurantiacus
b¹. Parasites.
Actinomyces phenotolerans

h. Cultures green or brownish-green.
Actinomyces viridochromogenes

i. Cultures black, producing a pigment of the melanin type.
Actinomyces niger

j. Cultures pigmented dark brown, but not black.
a¹. Saprophytes.
Actinomyces chromogenes
b¹. Plant parasites.
Actinomyces gracilis

2. Spores cylindrical or elongated.
a. Cultures colorless.
Actinomyces longisporus

b. Cultures red, sporophores mostly straight.
a¹. Saprophytes.
Actinomyces longisporus-ruber
b¹. Parasites.
a². Living in bodies of men and animals.
Actinomyces spumalis
b². Living on plants.
Actinomyces salmonicolor

c. Cultures orange.
Actinomyces fradiae

d. Cultures yellow.
a¹. Saprophytes.
Actinomyces longisporus-flavus
b¹. Parasites living on plants.
Actinomyces scabies

e. Cultures citron-yellow.
Actinomyces virgatus

f. Cultures green.
Actinomyces viridans

g. Cultures brown or chocolate-colored.
Actinomyces halstedii

h. Cultures black.
Actinomyces nigrificans

II. Sporophores straight or wavy, but not spiral.
1. Spores produced by means of fragmentation of plasma within cells.
a. Spores spherical or oval.
a¹. Cultures colorless.
Actinomyces globisporus

b¹. Cultures green.
a². Saprophytes.
Actinomyces viridis
b². Plant parasites.
Actinomyces cretaceus
c¹. Cultures brown.
Actinomyces globosus
b. Spores cylindrical or elongated.
a¹. Cultures colorless.
Actinomyces candidus
b¹. Cultures pigmented.
Actinomyces cylindrosporus
2. Spores produced by means of segmentation of aerial hyphae.
a. Cultures colorless.
Actinomyces farinosus
b. Cultures pigmented red.
Actinomyces oidiosporus
Actinomyces rectus
c. Cultures yellow-orange.
Actinomyces longissimus
d. Cultures blue.
Actinomyces caeruleus
e. Cultures brown.
Actinomyces fumosus

B. Sporophores produced in verticils.
I. Sporophores straight.
Actinomyces verticillatus
II. Sporophores spiral-shaped.
1. Spores spherical, oval.
Actinomyces reticuli
Actinomyces reticulus-ruber
2. Spores cylindrical, elongated.
Actinomyces circulatus

Detailed consideration was given to some of the larger groups of the genus, notably to the *Albus* and *Flavus* groups, as will be shown in Chapter 6.

5. WAKSMAN AND HENRICI SYSTEM (1943)

This system was used in the last two editions of Bergey's Manual of Determinative Bacteriology (1948, 1957). It may be listed here among the earlier systems. It was based primarily upon the ecology of the organisms, production of soluble pigments in organic and synthetic media, and proteolytic properties.

A. Saprophytes; psychophilic to mesophilic.
1. Soluble pigment on organic media other than brown, or faint brown.
a. Pigment absent or faint brown only.
b. Pigment blue.
c. Pigment at first green, becoming brown, etc.
2. Soluble pigment on organic media brown.
3. No soluble pigment produced in organic media.
a. Proteolytic action strong.
b. Proteolytic action limited.
c. Proteolytic action very weak.

B. Saprophytes; thermophilic.
1. Yellowish growth on potato; diastatic.
2. Dark-colored abundant growth on potato; nondiastatic.
3. Thermotolerant cultures.

C. Plant parasites or cultures isolated from diseased plants or from soil in which diseased plants were grown.
1. Isolated from potato scab or from soil in which scabby potatoes were grown.
2. Grown on or isolated from sweet potatoes.
3. Isolated from scab on mangels and sugar beets.

D. Isolates from animal tissues; in the animal body, hyphae often show clavate enlargements at the ends.
1. Limited proteolytic action.
2. Strong proteolytic action.

E. Produce only substrate growth and no aerial mycelium.

The last system, like the earlier ones, may now be considered as of purely historic interest. For the purpose of this treatise, it has been considerably modified. It has been greatly enlarged to include all newly described organisms belonging to the genus *Streptomyces*. Some of the features of the older system, notably those pertaining to ecology, have been left out altogether.

Recent Systems of Classification

Several systems for classifying species belonging to the genus *Streptomyces* have recently been proposed. Some of these have been selected for detailed examination. They are based largely upon morphology, cultural and biochemical properties, or combinations of these.

1. HESSELTINE, BENEDICT, AND PRIDHAM SYSTEM (1954)

In this system, emphasis was laid upon morphology as the basis for separation of the genus into five basic groups. After a study of hundreds of cultures on a variety of media, the conclusion was reached that the morphology of any one strain of *Streptomyces* is essentially the same on any medium where sporulation occurs. The major groups were subdivided into a number of subgroups on the basis of cultural properties, pigmentation of spores, and other criteria.

I. Sporophores not restricted in length, bearing fertile branches in verticils, with spores more or less strongly attached.
   1. Fertile branches in simple verticils, branches not ending in spirals.
   2. Fertile branches in simple verticils, branches ending in spirals.
   3. Fertile branches with compound verticils, branches not ending in spirals.

II. Sporophores with branches all straight, never ending in spirals; verticils absent.

III. Sporophores predominantly in tufts, never verticillate; outline of branches flexuous and irregular.

IV. Sporophores with branches ending in spirals, verticils being absent; sporophores either as long stalks bearing very short branches, or as short stalks bearing branches irregularly.
   1. Branches ending in open spirals with many turns.
   2. Branches ending in closed spirals with few turns, thus appearing as tight knots.

V. Sporophores with long and straight branches with spirals of large diameter at their ends; spirals usually with only a few turns, never verticillate.

No strains were observed in which the sporophores were unbranched, except when they were growing under unfavorable conditions or where degenerated type cultures were studied.

Seven major *Streptomyces* groups were thus created as indicated by the following key:

I. Sporophores produce verticils; spores not readily separating; aerial mycelium white, pink, lavender, or tan.
   Group I. *Streptomyces reticuli*
   1. Verticil branches simple.
      a. Sporophores straight.
      b. Sporophores spiral-shaped.
   2. Verticil branches compound.
      a. Ultimate branches straight.
      b. Ultimate branches spiraled.

II. Sporophores not producing any verticils; spores readily separate; color of aerial mycelium often not pink, white, lavender, or tan.
   1. Spirals always formed; color of aerial mycelium blue, blue-green, or green.
      Group II. *Streptomyces viridochromogenes*
   2. Spirals may or may not be formed; color of aerial mycelium different.
      a. Spirals never formed, tufts often present; color of aerial mycelium greenish-tan or tan, never white.
         Group III. *Streptomyces griseus*
      b. Spirals produced; color of aerial mycelium lavender, red, pink, or nearly tan.
         Group IV. *Streptomyces lavendulae*
   3. Sporophores straight.
      a. Spores white or nearly so.
         Group V. *Streptomyces albus*
      b. Aerial mycelium never white.
         a^1. Aerial mycelium yellow.
            Group VI. *Streptomyces parvus*
         b^1. Aerial mycelium gray, gray-brown, olive-gray, blackish-gray.
            Group VII. *Gray-spored group.*

It was suggested that the last group could

be subdivided on the basis of spiral formation.

2. FLAIG AND KUTZNER SYSTEM (1954, 1960)

Flaig and Kutzner (1954) and Kutzner (1956) studied about 2000 *Streptomyces* cultures, 63 of which were authentic species and the rest fresh soil isolates. They characterized their cultures by several criteria including cultural characteristics on complex and synthetic media, morphology of the aerial mycelium, physiological properties, and antibiotic activity against five test organisms. On the basis of these studies, a key was prepared. At first the material was divided into "groups" on the basis of the color of the aerial mycelium on oatmeal agar, six spore colors being recognized. The gray color group was further divided, on the basis of the color of the substrate mycelium and soluble pigment on this medium, thus resulting in the 10 groups shown in Table 9.

Later, however, Flaig and Kutzner (1960) reached the conclusion that the subdivision of the gray-spored color group may lead to difficulties when new isolates have to be placed in one of these groups, although this system proved to be quite useful in studying many strains at the same time.

Each group was further divided into subgroups (altogether 382) on the basis of pigment formation on glucose-peptone agar, morphology of the aerial mycelium, antibiotic activity against five test organisms, and cultural characteristics on several media. The shape of the spores (observed with electron microscope) was given for 175 of the 382 subgroups. Various authentic species were included in these subgroups according to their characteristic properties. The following species were placed in the various groups.

Group I: *S. albus*, *S. griseus*, *S. chrysomallus*, *S. coelicolor*, *S. californicus*

Group II: *S. longispororuber*, *S. bobiliae*, *S. roseochromogenes*, *S. venezuelae*, *S. phaeochromogenes*

Group III: *S. lavendulae*, *S. xanthophaeus*

Group IV: *S. flavogriseus*, *S. globisporus*, *S. flaveolus*

Group V: *S. diastaticus*, *S. globosus*

Group VI: *S. flavus*

Group VII: *S. craterifer*, *S. griseolus*, *S. halstedii*, *S. hygroscopicus*, *S. aureofaciens*, *S. violaceoruber*

Group VIII: *S. purpurascens*

Group IX: *S. cyaneus*, *S. viridochromogenes*, *S. chartreusis*

Group X: *S. hirsutus*, *S. prasinus*, *S. prasinopilosus*

TABLE 9

Grouping of the genus Streptomyces *according to characteristic coloration* (Flaig and Kutzner, 1960)

Group	Aerial mycelium	Substrate mycelium and soluble pigment
I	Yellowish to yellow-gray	Colorless, brownish, reddish, greenish
II	Light rose to reddish	Colorless, orange, greenish, brownish to dark brown, pink to dark red
III	Gray-rose (lavender)	Colorless, orange, brownish to dark brown
IV	Light gray to gray	Yellowish-green to green
V	Gray	Brown
VI	White to gray (cottony)	Colorless, brownish to brown
VII	Gray (dusty)	Colorless, greenish-gray, brownish-gray, orange-brownish, red or blue violet
VIII	Light gray or pink	Violet
IX	Blue	Blue-purple, bluish-green, brownish
X	Green	Colorless, greenish, brownish to rose-red

3. SYSTEM OF YAMAGUCHI AND SABURI (1955)

Yamaguchi and Saburi also used morphological features as the primary basis for the separation of the genus *Streptomyces* into groups, and physiological characteristics for further separation. Although they were concerned primarily with species of *Streptomyces* possessing antitrichomonal properties, their system may apply to the genus as a whole.

I. Sporophores straight, tuft-forming tendency in the margin; no verticils or spirals.
  1. Aerial mycelium gray.
    a. Soluble pigment on protein media. Light purple, reddish-purple, purplish-brown, sometimes yellowish-brown.
      Streptomyces purpeofuscus
    b. No soluble brown pigment.
      Streptomyces fasciculus
  2. Aerial mycelium pale yellowish-green.
    a. Soluble pigment on protein media brown—ATCC Culture No. 3309.
    b. Soluble pigment brown.
      Streptomyces griseus

II. Sporophores straight, verticils produced.
  1. Cottony aerial mycelium white, light tan, or pale pink.
    a. Soluble pigment on protein media brown.
      Streptomyces reticuli
    b. No soluble brown pigment on protein media.
      Streptomyces hachijoensis

III. Sporophores spiral-shaped.
  1. Predominantly closed spirals produced.
    a. Aerial mycelium gray.
      a^1. Soluble brown pigment on protein media.
        a^2. Growth colorless, yellowish-brown, brown, or deep brown.
          Streptomyces olivochromogenes
        b^2. Growth light purple to purplish-black.
          Streptomyces purpureochromogenes
      b^1. No soluble brown pigment.
        Streptomyces aureofaciens
    b. Aerial mycelium pale pink.
      a^1. Soluble pigment on protein media brown.
        Streptomyces lavendulae
      b^1. No soluble brown pigment.
        Streptomyces fradiae
  2. Predominantly open spirals or compact spirals produced.
    a. Aerial mycelium white.
      a^1. Soluble pigment on protein media brown.
        a^2. Growth reddish.
          Streptomyces ruber
      b^1. No soluble brown pigment.
        Streptomyces farinosus
    b. Aerial mycelium gray.
      a^1. Soluble pigment on protein media brown.
        a^2. Abundant compact spirals produced on aerial hyphae.
          Streptomyces naganishii
        b^2. Growth colorless, white, light yellow, or yellowish-brown.
          Streptomyces diastatochromogenes
        c^2. Growth colorless, reddish-orange, or reddish-purple.
          Streptomyces griseoruber
      b^1. No soluble brown pigment.
        a^2. Growth colorless to creamy.
          Streptomyces albus
        b^2. Growth colorless, white, light yellow, or yellowish-brown.
          G 167 (resembling *Streptomyces cacaoi*)
        c^2. Growth colorless, light yellow, or light pinkish-brown.
          Streptomyces albogriseolus
        d^2. Growth purple to pink to red.
          Streptomyces californicus
    c. Aerial mycelium gray, but on certain media moist with dark, glistening patches.
      Streptomyces hygroscopicus

IV. No characteristic features of aerial hyphae.
  1. Very limited aerial mycelium production on various media.
    a. Soluble pigment on protein media deep brown.
      a^1. Growth yellow to yellowish-brown.
        Streptomyces flavochromogenes
    b. Soluble pigment faint yellowish-brown.
      Streptomyces thioluteus
  2. Aerial mycelium white.
    a. Growth colorless, light yellow, or reddish-orange.
      Streptomyces ruber

4. BALDACCI SYSTEM (1956, 1958, 1959)

Following the example of Sanfelice and Waksman, Baldacci divided the genus

Streptomyces into sections, based upon the color of the substrate mycelium. Each section was divided into series, on the basis of the color of the aerial mycelium. Each series was divided into species.

The genus was characterized by the presence or absence of spores, the arrangement of spores, and the ramification and breaking up of the substrate mycelium. The species were characterized by enzymatic reactions, antibiotic activity, and soluble pigments spreading through the substratum, depending on nutrition and pH.

Although Baldacci discussed the genus under the name "*Actinomyces*," he actually meant *Streptomyces*, without recognizing it as such, since he made no mention of other species and other genera.

A. *Actinomycetes cum sporophora solitaria vel congregata.*

Section I. Substrate mycelium colorless; scant development on agar, showing a veiled cobweb-like appearance; limited sporulation.

1. Aerial mycelium white.
   Series *Albus*
2. Aerial mycelium sea-green.
   Series *Griseus*
3. Aerial mycelium green-azure.
   Series *Viridis*
4. Aerial mycelium azure.
   Series *Caeruleus*
5. Aerial mycelium white-wine-lavender.
   Series *Lavendulae*
6. Aerial mycelium light pink.
   Series *Roseus*
7. Aerial mycelium gray.
   Series *Diastaticus*

Section II. Substrate mycelium colored; development generally abundant on agar (creamy, lichenoid, etc.); delayed or partial sporulation.

(a) Substrate mycelium yellow to yellow-brown.

9. Aerial mycelium white.
   Series *Albidoflavus*
10. Aerial mycelium pink (white to pink).
    Series *Roseoflavus*
11. Aerial mycelium yellow with gray spots.
    Series *Flavus*
12. Aerial mycelium grayish.
    Series *Aureus*

(b) Substrate mycelium yellow to green-yellow.

13. Aerial mycelium gray-white.
    Series *Flavoviridis*
14. Aerial mycelium white to lemon-yellow.
    Series *Virgatus*

(c) Substrate mycelium yellow with green and pinkish spots.

15. Aerial mycelium white to pink.
    Series *Madurae*

(d) Substrate mycelium brown to black.

16. Aerial mycelium white to gray.
    Series *Scabies*
17. Aerial mycelium red.
    Series *Roseochromogenes*
18. Aerial mycelium yellow.
    Series *Sulphureus*
19. Aerial mycelium gray.
    Series *Antibioticus*
20. Aerial mycelium grayish-flesh-colored.
    Series *Griseoincarnatus*

(e) Vegetative mycelium brown to green-brown.

21. Aerial mycelium gray.
    Series *Intermedius*

(f) Vegetative mycelium brown.

22. Aerial mycelium white to leather-brown.
    Series *Rimosus*

(g) Vegetative mycelium orange.

23. Aerial mycelium seashell pink.
    Series *Fradiae*

(h) Vegetative mycelium flesh-rose.

24. Aerial mycelium white.
    Series *Bostroemi*

(i) Vegetative mycelium red.

25. Aerial mycelium white to pink.
Series *Albosporeus*
26. Aerial mycelium ash-gray.
Series *Cinereo-ruber*

(j) Vegetative mycelium violet-blue-red.
27. White to gray aerial mycelium with different shades.
Series *Violaceus*

B. *Actinomycetes cum sporophora opposita et verticillata**

Section I. Substrate mycelium colorless.
1. Aerial mycelium white or whitish.
Series *Circulatus*
2. Aerial mycelium gray and pinkish.
Series *Griseocarneus*

Section II. Vegetative mycelium colored.

(a) Substrate mycelium lemon-yellow-creamy-colored.
3. Aerial mycelium cinnamon-colored.
Series *Cinnamoneus*

(b) Substrate mycelium brownish-yellow.
4. Aerial mycelium gray.
Series *Reticuli*

(c) Substrate mycelium brown.
5. Aerial mycelium greenish-gray.
Series *Verticillatus*

(d) Substrate mycelium red.
6. Aerial mycelium pinkish-red.
Series *Rubrireticuli*

5. GAUSE *ET AL.* SYSTEM (1957)

Gause *et al.* modified Baldacci's system, leaving out the sections and combining the pigmentation of the aerial mycelium with that of the substrate growth for series characterization. Each series was subdivided, on the basis of formation of a soluble pigment in a complex organic medium, or of the structure of the sporophores, or of the pigmentation of a synthetic medium. These investigators, like Baldacci, adhered to the genus designation *Actinomyces*, without, however, considering the accumulated information concerning all other genera. Little consideration was given to previously named species.

I. Aerial mycelium rose-purple, substrate mycelium colorless.
Series *Lavendulae-roseus*

II. Aerial mycelium rose-colored; substrate mycelium yellow.
Series *Fradiae*

III. Aerial mycelium rose-colored; substrate mycelium brown.
Series *Fuscus*

IV. Aerial mycelium light rose; substrate mycelium violet:
Series *Roseoviolaceus*

V. Aerial mycelium rose-colored; substrate mycelium red.
Series *Ruber*

VI. Aerial mycelium yellowish-green or cream-colored; substrate mycelium colored or colorless.
Series *Helvolus*

VII. Aerial mycelium white; substrate mycelium colorless.
Series *Albus*

VIII. Aerial mycelium white; substrate mycelium red or brown.
Series *Albosporeus*

IX. Aerial mycelium blue or greenish-blue; substrate mycelium colorless or blue-colored.
Series *Coerulescens*

X. Aerial mycelium gray; substrate mycelium colorless.
Series *Griseus*

XI. Aerial mycelium gray, then black (result of autolysis); substrate mycelium colorless.
Series *Nigrescens*

XII. Aerial mycelium gray; substrate mycelium yellow or orange.
Series *Aureus*

XIII. Aerial mycelium gray; substrate mycelium yellow-brown.
Series *Chrysomallus*

XIV. Aerial mycelium gray; substrate mycelium brown-black.
Series *Chromogenes*

* These sections were placed by Baldacci in a separate genus "*Streptoverticillium*."

XV. Aerial mycelium gray; substrate mycelium blue-violet or red-brown.
Series *Violaceus*

6. PRIDHAM, HESSELTINE, AND BENEDICT SYSTEM (1957, 1958)

In their earlier system, these investigators divided the genus *Streptomyces* into seven groups. Each group was characterized by a distinct morphology of the sporophores in mature cultures, and by a distinct color of the aerial mycelium. This system subsequently was revised. Morphological sections and color series were established and, on the basis of literature study and laboratory investigations, many species and known antibiotic-producing strains were cataloged. It was suggested that evaluation of the component strains in the sections and series, by physiological tests, would allow the determination of ranges of variation and a more logical approach to speciation in the genus. The placement by these investigators of strains in morphological sections, regardless of species designation, has suggested synonymy, as well as misidentification of many strains.

The following bases were considered in justifying these subdivisions:

1. The morphology of the sporophores of a particular strain does not appreciably change on substrata that support optimal formation of aerial mycelium, sporophores, and spores. Morphological patterns exhibited by streptomycetes are not subject to considerable variation, unless degeneration of a particular strain has occurred through improper maintenance. Morphological examinations should be made after two weeks' incubation at 28–30°C on several appropriate media.

2. The color of the sporulating aerial mycelium of a given strain at maturity was said not to differ appreciably from medium to medium. Each morphological section of the genus can be further subdivided into color "series." Each color series can be subdivided, on the basis of physiological criteria, into "species." Additional delineation can then be used to create "varieties" or "physiological forms," if need be.

3. The present concept of the genus *Streptomyces* is interpreted rather broadly. Some of the strains identified as members of the genus may in reality belong to other genera.

The proposed sections were designated as follows:

I. *Rectus-flexibilis* (RF). Sporophores straight, flexuous, or fascicled; no spirals. Type species *S. griseus*.

II. *Retinaculum-apertum* (RA). Sporophores in the form of hooks, open loops, or greatly extended spirals. Type species *S. fradiae*.

III. *Spira* (S). Sporophores either short and gnarled, or in the form of compact spirals or of extended long and open spirals. Type species *S. viridochromogenes* (Fig. 26).

IV. *Monoverticillus* (MV). Sporophores in the form of primary verticils attached to long, straight branches; no spirals.

V. *Monoverticillus-spira* (MV-S). Sporophores as primary verticils attached to long, straight branches; elements of verticils spiraled.

VI. *Biverticillus* (BV). Sporophores as compound verticils attached to long, straight branches; no spirals. Type species *S. cinnamomeus* f. *cinnamomeus*.

VII. *Biverticillus-spira* (BIV-S). Sporophores as compound verticils attached to long, straight branches; elements of secondary verticils spiraled.

In addition to the above sections, another section was set up to include strains for which no micromorphological data were available.

Each "section" was subdivided into "series" based on the color of sporulating aerial mycelium at maturity. The proposed series were designated as follows:

1. White.

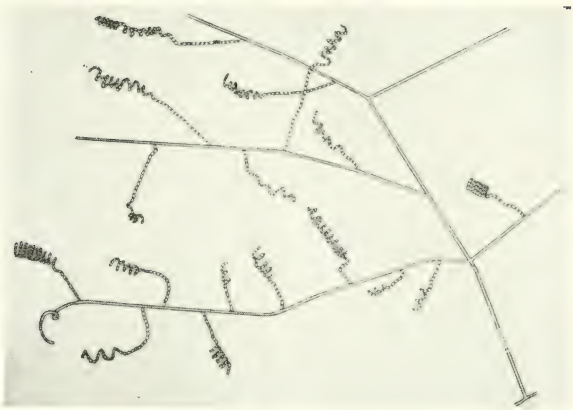

FIGURE 26. Spiral formation by *Streptomyces* 240 (Reproduced from: Naganishi, H. and Nomi, R. J. Fermentation Technol. 32: 492, 1954).

2. Olive-buff (buff to tan to olive-buff).
3. Yellow.
4. Blue (blue to blue-green to green).
5. Red (pink to red to lavender to lavender-gray).
6. Gray (light gray to mouse-gray to brown-gray to gray-brown).

An additional "unknown" series was set up to include strains for which no color data were available.

7. ETTLINGER, CORBAZ, AND HÜTTER SYSTEM (1958)

Ettlinger *et al.* considered four major characters of *Streptomyces* that were stable and reliable enough to justify their system of classification. These characters were: (a) morphology of the spores, (b) color of aerial mycelium, (c) morphology of aerial mycelium, and (d) formation of melanoid pigment.

These investigators suggested combination of sections 4 and 6, and sections 5 and 7 of the Pridham *et al.* (1958) system, since they had never observed nonbranching verticils. They recognized a total of 15 morphological types distributed among the five sections. They also recognized the following color groups for the aerial mycelium: (1) *niveus* (snow-white), (2) *griseus* (yellowish- to greenish-gray), (3) *azureus* (sky-blue), (4) *cinnamoneus* (light carmine to brownish), (5) *cinereus* (ash-gray), (6) *prasinus* (leek-green).

They observed certain constant relationships among some of the four basic properties. The *griseus* and *cinnamoneus* color groups were found to occur only in strains with smooth spores. The *azureus* and *prasinus* color groups occurred only in strains with spiny or hairy spores. The latter always were found associated with the occurrence of spirals.

Other properties, such as soluble pigment on synthetic media, antibiotic activity, and pigmentation of substrate mycelium, were found to be variable. Gelatin liquefaction, milk coagulation, starch hydrolysis, and other physiological properties were not considered of great value from a systematic point of view, since no true negative gelatin liquefaction or negative starch hydrolysis was ever detected.

On the basis of the above properties, the

following system of classification was proposed for the genus *Streptomyces:*

A. Spores spiny or hairy.
  I. Aerial mycelium blue.
    1. *Streptomyces viridochromogenes*
  II. Aerial mycelium not blue.
    1. Aerial mycelium white.
      2. *Streptomyces purpurascens*
    2. Aerial mycelium not white.
      a. Aerial mycelium green.
        a^1. Spores with short spines.
          3. *Streptomyces prasinus*
        b^1. Spores with longer spines or with hair.
          a^2. Spores with stiff spines.
            4. *Streptomyces hirsutus*
          b^2. Spores with flexible hair.
            5. *Streptomyces prasinopilosus*
      b. Aerial mycelium gray.
        a^1. Sporophores in verticils.
          a^2. Sporophores in open spirals; no melanin formation.
            6. *Streptomyces noursei**
          b^2. Sporophores in closed spirals; melanin produced.
            7. *Streptomyces echinatus**
        b^1. Sporophores not in verticils.
          a^2. Sporophores in closed spirals.
            8. *Streptomyces albogriseolus*
          b^2. Sporophores in open spirals.
            a^3. Spirals irregular.
              9. *Streptomyces macrosporeus*
            b^3. Spirals regular.
              a^4. Spores spiny.
                10. *Streptomyces griseoflavus*
              b^4. Spores hairy.
                a^5. Spirals with >5 turns; melanin-positive.
                  11. *Streptomyces pilosus*

FIGURE 27. Sporogenous coiled hyphae of *Streptomyces* T 3110; taken from a gray area of a colony (Reproduced from: Duggar, B. M. *et al.* Ann. N. Y. Acad. Sci. **60:** 71–85, 1954).

                b^5. Spirals with <5 turns, melanin-negative.
                  12. *Streptomyces flaveolus*

B. Spores smooth.
  I. Aerial mycelium yellowish- to greenish-gray.
    1. Melanin-negative.
      13. *Streptomyces griseus*
    2. Melanin-positive.
      14. *Streptomyces michiganensis*
  II. Aerial mycelium white.
    1. Sporophores in verticils.
      15. *Streptomyces rubrireticuli*
    2. No verticils produced.
      a. Sporophores form spirals.
        16. *Streptomyces niveoruber*
      b. Sporophores straight or wavy.
        a^1. Melanin-negative.
          17. *Streptomyces fulvissimus*
        b^1. Melanin-positive.
          18. *Streptomyces phaeochromogenes*

* The verticillate nature of these organisms is open to question.

III. Aerial mycelium light carmine to brownish.
1. Sporophores in verticils.
a. Sporophores straight or wavy.
19. *Streptomyces netropsis*
b. Sporophores in spirals.
20. *Streptomyces tendae*
2. Sporophores not in verticils.
a. Sporophores straight.
21. *Streptomyces venezuelae*
b. Sporophores in spirals.
a¹. Spirals at end of long, straight sporophores; melanin-positive.
22. *Streptomyces lavendulae*
b¹. Spirals different; melanin-negative.
a². Spirals closed.
23. *Streptomyces violaceoniger*
b². Spirals open.
a³. Spirals regular, usually >5 turns.
24. *Streptomyces fradiae*
b³. Spirals irregular, usually <5 turns.
25. *Streptomyces erythraeus*
IV. Aerial mycelium ash-gray.
1. Sporophores in verticils.
26. *Streptomyces reticuli*
2. Sporophores not in verticils.
a. Sporophores straight or wavy.

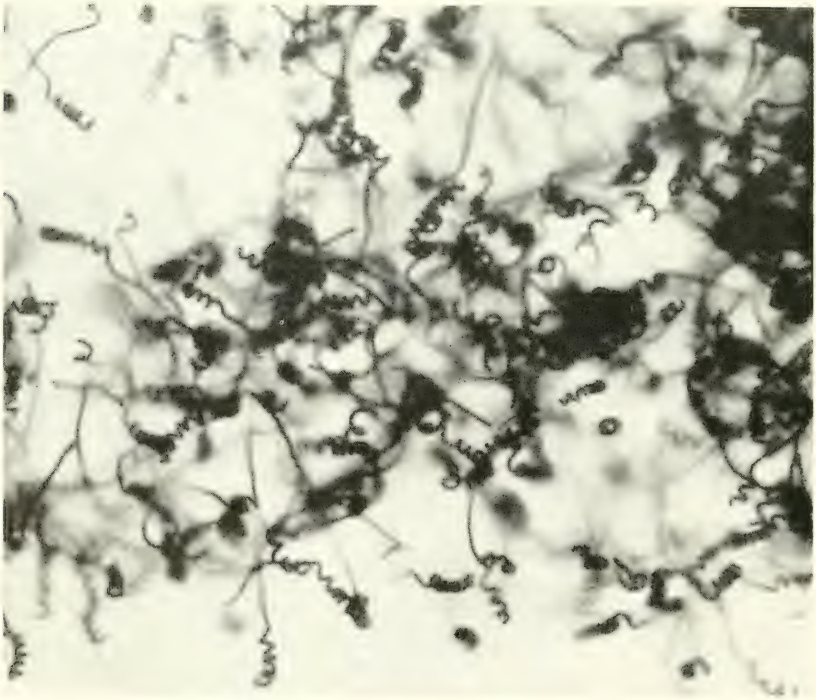

FIGURE 28. Sporogenous coiled hyphae of *Streptomyces* T 3110; taken from a blue sector of a colony (Reproduced from: Duggar, B. M. *et al.* Ann. N. Y. Acad. Sci. **60**: 71–85, 1954).

a¹. Sporophores sympodially branched.
27. *Streptomyces viridogenes*
b¹. Sporophores monopodially branched.
a². Sporophores as side branches of sterile hyphae.
28. *Streptomyces ramulosus*
b². Sporophores different.
a³. Melanin-negative.
29. *Streptomyces olivaceus*
b³. Melanin-positive.
30. *Streptomyces antibioticus*
b. Sporophores in spirals.
a¹. Spirals closed.
31. *Streptomyces hygroscopicus*
b¹. Spirals open.
a². Spirals irregular, usually >5 turns.
32. *Streptomyces aureofaciens*
b². Spirals regular, <5 turns.
a³. Melanin-negative.
33. *Streptomyces parvullus*
b³. Melanin-positive.
34. *Streptomyces galilaeus*

8. SHINOBU SYSTEM (1958b)

Shinobu proposed the following system for grouping of the species of the genus *Streptomyces:*

Group I. Monopodial branching, straight or wavy aerial mycelium; never producing spirals.
Subgroup 1. Tyrosinase reaction: positive; nitrite production: positive.
Streptomyces olivaceus
Subgroup 2. Tyrosinase reaction: positive; nitrite production: negative.
Streptomyces phaeopurpureus
Subgroup 3. Tyrosinase reaction: negative; nitrite production: positive.
Streptomyces sp. No. 2
Subgroup 4. Tyrosinase reaction: negative; nitrite production: negative.
Streptomyces sp. No. 232
Group II. Spiral formation; long or short, loose or compact, and open or closed spirals.
Subgroup 5. Tyrosinase reaction: positive; nitrite production: positive.
Streptomyces viridochromogenes
Subgroup 6. Tyrosinase reaction: positive; nitrite production: negative.
Streptomyces sp. No. 2076.
Subgroup 7. Tyrosinase reaction: negative; nitrite production: positive.
Streptomyces sp. No. 236
Subgroup 8. Tyrosinase reaction: negative; nitrite production: negative.
Streptomyces scabies
Group III. Verticil formation; primary and secondary verticils; rarely one tertiary verticil (Fig. 29).
Subgroup 9. Tyrosinase reaction: positive; nitrite production: positive.
Streptomyces hiroshimensis
Subgroup 10. Tyrosinase reaction: positive; nitrite production: negative.
Streptomyces luteoverticillatus
Subgroup 11. Tyrosinase reaction: negative; nitrite production: positive.
Streptomyces roseoverticillatus
Subgroup 12. Tyrosinase reaction: negative; nitrite production: negative.
Streptomyces olivoverticillatus
Group IV. Intermediate group of Nitella-type and Anitella-type verticil.
Subgroup 13.
Streptomyces spiroverticillatus

Shinobu further emphasized that in the identification of species of *Streptomyces* other characteristics should be considered. These are the following:

1. Morphological properties: type of colony, shape of spiral, etc.
2. Physiological properties: cellulase, mannase, and amylase reactions; utilization of carbon and nitrogen sources, etc.
3. Cultural properties: growth of colony, production of pigment, etc.

9. FROMMER SYSTEM (1959)

This system does not apply to the genus *Streptomyces* as a whole but only to the actinomycin-producing species.

A. Chromogenic group.
I. Spirals not formed on aerial mycelium. Occasionally a few spirals are found.

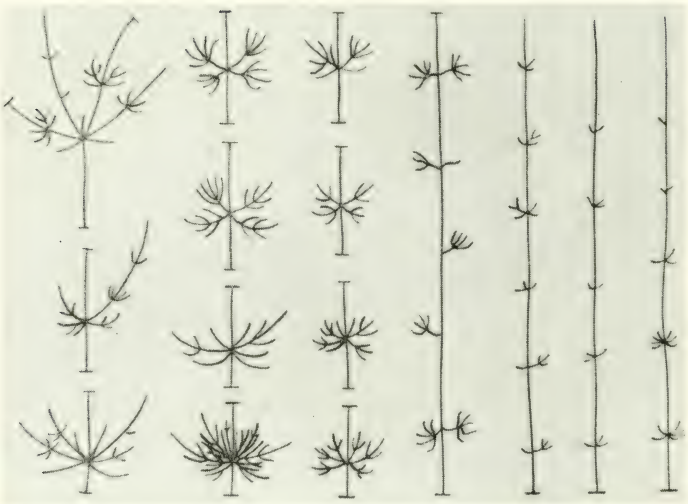

FIGURE 29. Verticil formation (Nitella type) including both primary and secondary verticils (Reproduced from: Shinobu, R. Mem. Osaka Univ. Lib. Arts and Ed. B. Nat. Sci. 7, 1958).

1. Aerial mycelium on synthetic agar media gray. Tyrosinase-negative.
   Streptomyces antibioticus
2. Aerial mycelium on synthetic agar media yellow. Tyrosinase-positive.
   Streptomyces michiganensis

II. Numerous spirals produced on aerial mycelium.
1. Yellow or yellow-green pigment produced on synthetic agar.
   Streptomyces galbus
2. Soluble pigment on synthetic agar, dark brown.
   Streptomyces lanatus

B. Nonchromogenic group.
I. No spirals on aerial mycelium.
1. Strongly proteolytic. Aerial mycelium on synthetic agar white, yellow, or greenish.
   Streptomyces chrysomallus
2. Weakly proteolytic. Aerial mycelium on synthetic agar mouse-gray.
   Streptomyces chrysomallus v. *fumigatus*

II. Numerous spirals on aerial mycelium.
1. Aerial mycelium on glycerol-glycine agar grayish-rose. Practically no growth on synthetic agar.
   Streptomyces murinus
2. Abundant growth on synthetic agar. Aerial mycelium on glycerol-glycine agar cream-colored.
   Streptomyces galbus v. *achromogenes*

10. MAYAMA SYSTEM (1959)

Mayama (1959) concluded that morphology, serological reactions, and types of growth on liquid media are the most important properties for the classification of *Streptomyces*. On the basis of these properties, he divided the genus into seven groups: *S. olivaceus*, *S. lavendulae*, *S. aureofaciens*, *S. griseolus*, *S. albus*, *S. rimosus*, and *S. reticuli*. In addition to these, he also listed a number of species for which no group characteristics were known, notably *S. antibioticus*, *S. fulvissimus*, *S. ruber*, *S. coelicolor*, etc.

Mayama (1959) and Mayama and Tawara (1959) classified the genus *Streptomyces* into five sections and 14 series:

Section I. Aerial mycelium irregularly branched. Sporophores produced at the

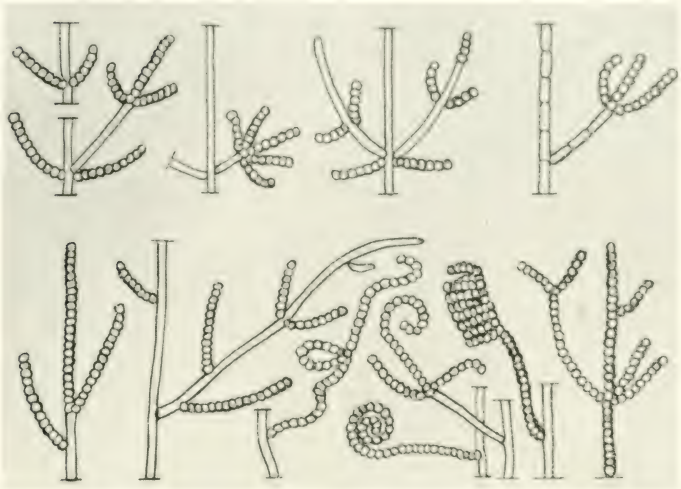

FIGURE 30. Spore formation in *Streptomyces* species (Reproduced from: Shinobu, R. Mem. Osaka Univ. Lib. Arts and Ed. B. Nat. Sci. 7, 1958).

terminal portion of the branching hyphae.
Series 1. Sporophores straight to flexuous.
Series 2. Sporophores form open loops.
Series 3. Sporophores form spirals.
Section II. Aerial mycelium branches in tuft formations. Sporophores produced at the terminal portion of the branching hyphae.
Series 4. Sporophores straight to flexuous.
Series 5. Sporophores form open loops.
Series 6. Sporophores form spirals.
Section III. Aerial mycelium forms long main stem. Sporophores produced at the terminal portion of side branches. Nonverticillate.
Series 7. Sporophores straight to flexuous.
Series 8. Sporophores form open loops.
Series 9. Sporophores form spirals.
Section IV. Aerial mycelium forms long main stem. Sporophores produced at terminal portion of side branches. Verticillate.
Series 10. Monoverticillate, straight to flexuous.
Series 11. Monoverticillate, spirals.
Series 12. Biverticillate, straight to flexuous.
Series 13. Biverticillate, spirals.
Section V.
Series 14. No aerial mycelium.

11. NOMI SYSTEM

Finally a purely morphological system may be listed. Nomi (1959) proposed a division of the genus *Streptomyces* into eight morphological groups. He returned to an earlier concept of Drechsler (1919) that the nature of the turn of the spirals, namely sinistrorse and dextrorse, is an important characteristic of *Streptomyces* species. He recognized, however, that some cultures may be rather indefinite in this respect.

A. Aerial hyphae somewhat flexuous or straight; few long hyphae. The terminal filaments develop into spiral-shaped sporophores.
1. Spirals sinistrorse.

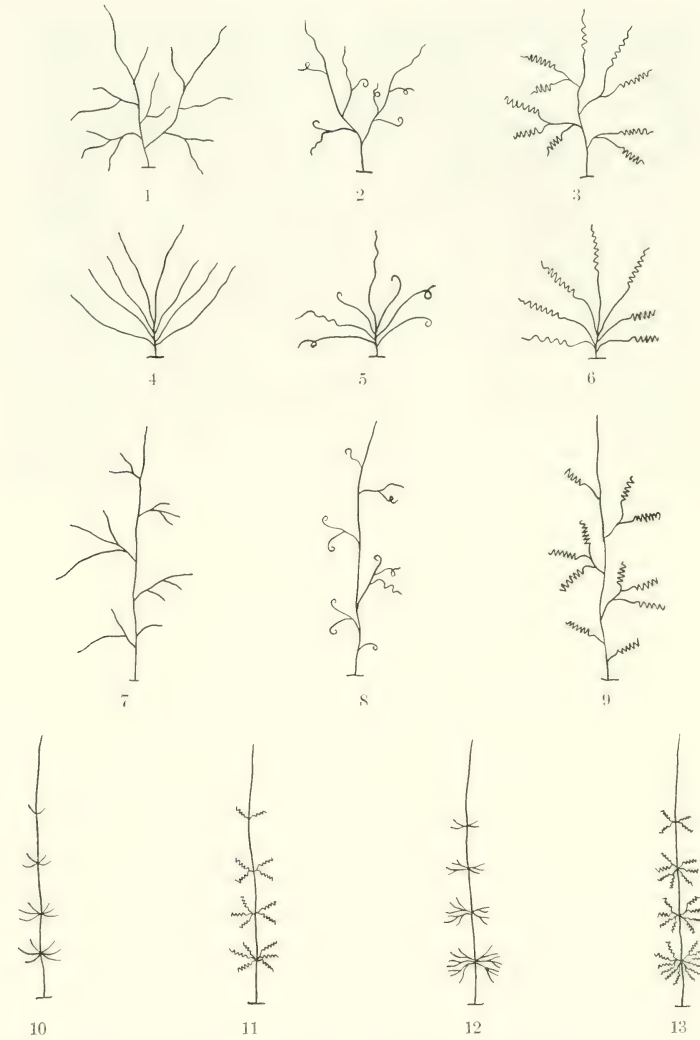

PLATE III. Morphological groups in the genus *Streptomyces* (Mayama, 1959) (For details, see text, pp. 102–103).

Section I: series 1—3
Section II: series 4—6
Section III: series 7—9
Section IV: series 10—13

a. Spirals long, extended to compact.
S. coelicolor, *S. albogriseolus*, *S. flavovirens*, *S. parvullus*
b. Spirals compact to compressed. None found.
2. Spirals dextrorse.
a. Spirals long, extended to compact.
S. viridochromogenes
b. Spirals compact to compressed.
Streptomyces sp. No. 189

B. Most aerial hyphae long, straight or slightly flexuous. They do not sporulate, but give rise to short side branches whose terminal filaments develop into spiral-shaped sporophores.
1. Spirals sinistrorse.
S. purpurascens
2. Spirals dextrorse.
Various unidentified forms.

C. Aerial hyphae irregularly flexuous or wavy; long hyphae absent. Terminal filaments form spirals.
1. Spirals sinistrorse.
S. sulphureus
2. Curvature of spiral indefinite.
S. griseoluteus

D. Aerial hyphae long, straight, or wavy. They give rise to short side branches, which develop into spore-bearing hyphae containing spirals.
1. Spirals sinistrorse.
S. hygroscopicus, *S. violaceoniger*, *S. albus*

E. Aerial hyphae in clusters. The terminal filaments develop into sporophores, both spiral and nonspiral forming.
S. vinaceus, *S. microflavus*, *S. fradiae*, *S. lavendulae*, *S. virginiae*, *S. cinnamonensis*, *S. roseochromogenes*, *S. phaeochromogenes*

F. Aerial hyphae branch in clusters. No spirals or loops.
S. venezuelae, *S. tanashiensis*, *S. bikiniensis*, *S. antibioticus*, *S. aureofaciens*, *S. olivaceus*, *S. nitrosporeus*, *S. griseus*, *S. lipmanii*, *S. rutgersensis*, *S. parvus*, *S. flavovirens*, *S. californicus*, *S. vinaceus*, *S. ruber*, *S. caeruleus*

G. Aerial hyphae long, straight or slightly flexuous. Verticillate. No spirals.
S. reticuli, *S. griseocarneus*, *S. echimensis*, *S. hiroshimensis*, *S. salmonicida*, *S. thioluteus*, *S. albireticuli*, *S. netropsis*

H. Aerial hyphae somewhat flexuous or wavy. Long hyphae and spirals are not produced.
S. albus (atypical), *S. halstedii*, *S. scabies*, *S. verne*, *S. griseolus*, *S. erythreus* (Plates IV and V)

12. OTHER SYSTEMS

Other systems have been proposed for the classification of the genus *Streptomyces*. Some of these systems are modifications or supplementations of that presented in Bergey's Manual (7th ed., 1957), or modifications of one or the other of those outlined in this chapter.

One of these is the system outlined by Routien (1959). The various species included in the genus *Streptomyces* were divided into three major groups: (1) saprophytes; (2) plant parasites or cultures isolated from diseased plants or from soil in which diseased plants were grown; (3) cultures isolated from animal tissues. These groups were subdivided on the basis of formation and color of aerial mycelium (green, brownish-purple to black, blue-gray or blue-green, yellowish to orange, pink to rose, etc.). The color of the substrate mycelium and the various biochemical properties were then used for further subdivisions. Morphology (spiral formation, shape of spores) played only a minor role in this system. See also Sakai, 1959.

Summary of the Properties Used in Subdividing the Genus Streptomyces

Evaluation of the above systems of classification leads to the conclusion that sporophore morphology has been given first or second consideration by the great majority of investigators. Lesser attention was paid to the color of the aerial mycelium and the nature of soluble pigments. Chromogenesis, or pigment formation in protein media, was often given first position. Antibiotic production and ecology received the least consideration.

In Baldacci's system of dividing the genus into groups or series, the color of the sub-

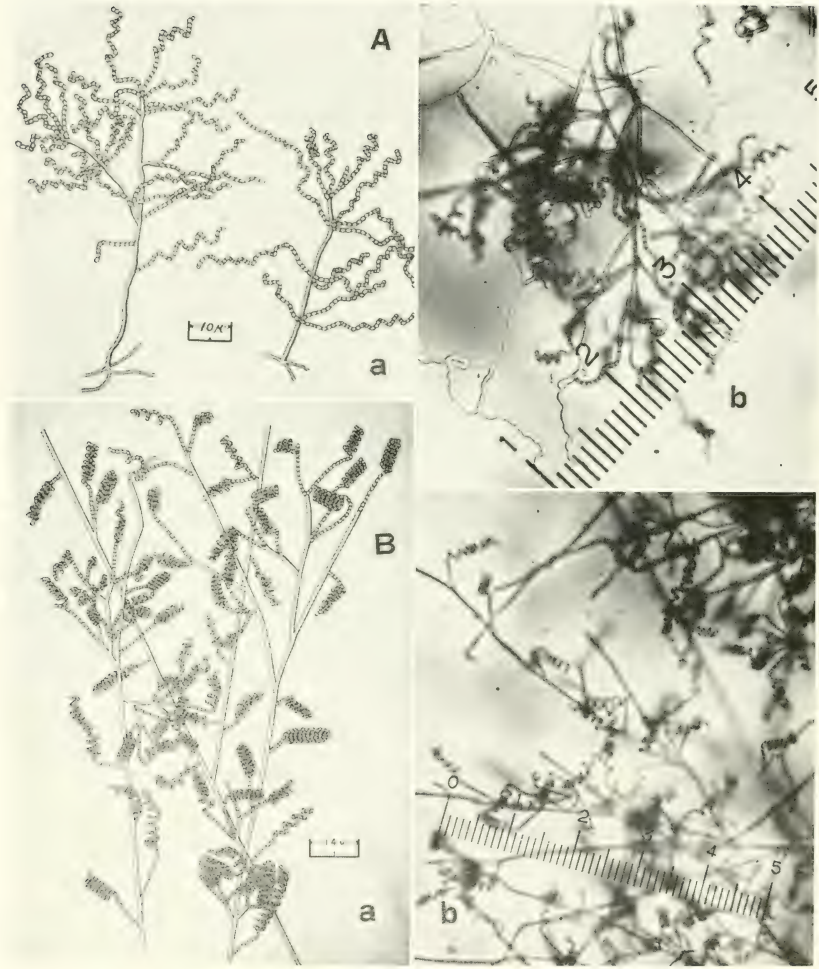

PLATE IV. Morphological types, according to Nomi (1959). *a*. represents a schematic presentation of each type; *b*. gives the actual photograph.

A. Aerial hyphae flexuous or straight; spirals extended to compact (*S. coelicolor*).

B. Aerial hyphae straight; spirals on side branches (*S. purpurascens*).

(*See continuation, next plate*).

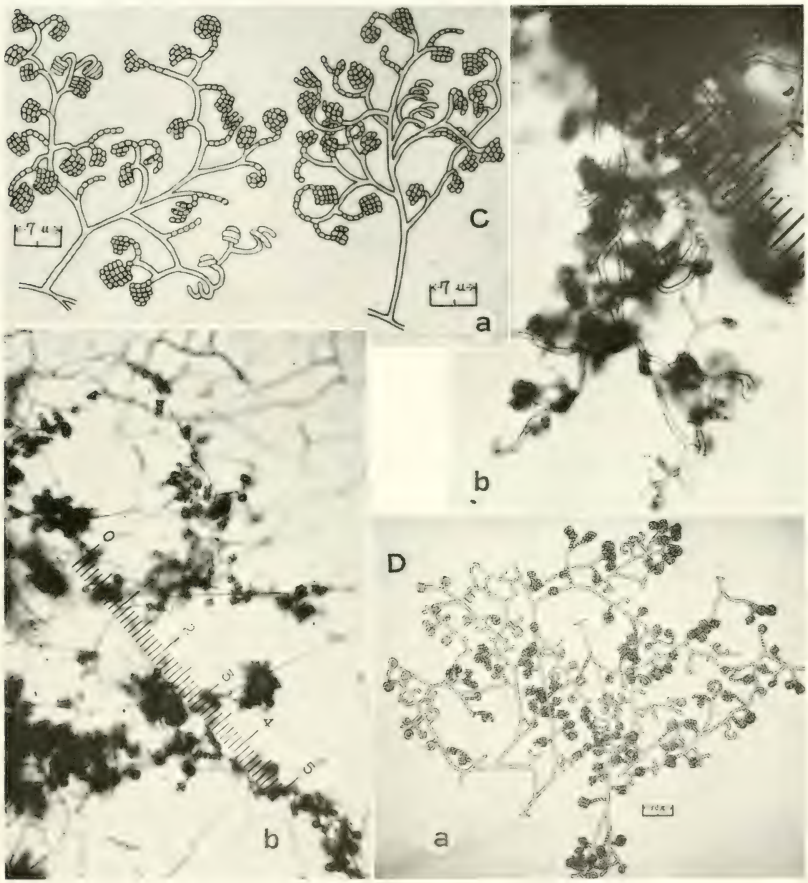

PLATE IV. (*Continued*)

C. Aerial hyphae wavy; spirals on terminal filaments (*S. griseoluteus*).

D. Aerial hyphae long, straight or wavy; sporophores as side branches; spirals produced (*S. hygroscopicus*).

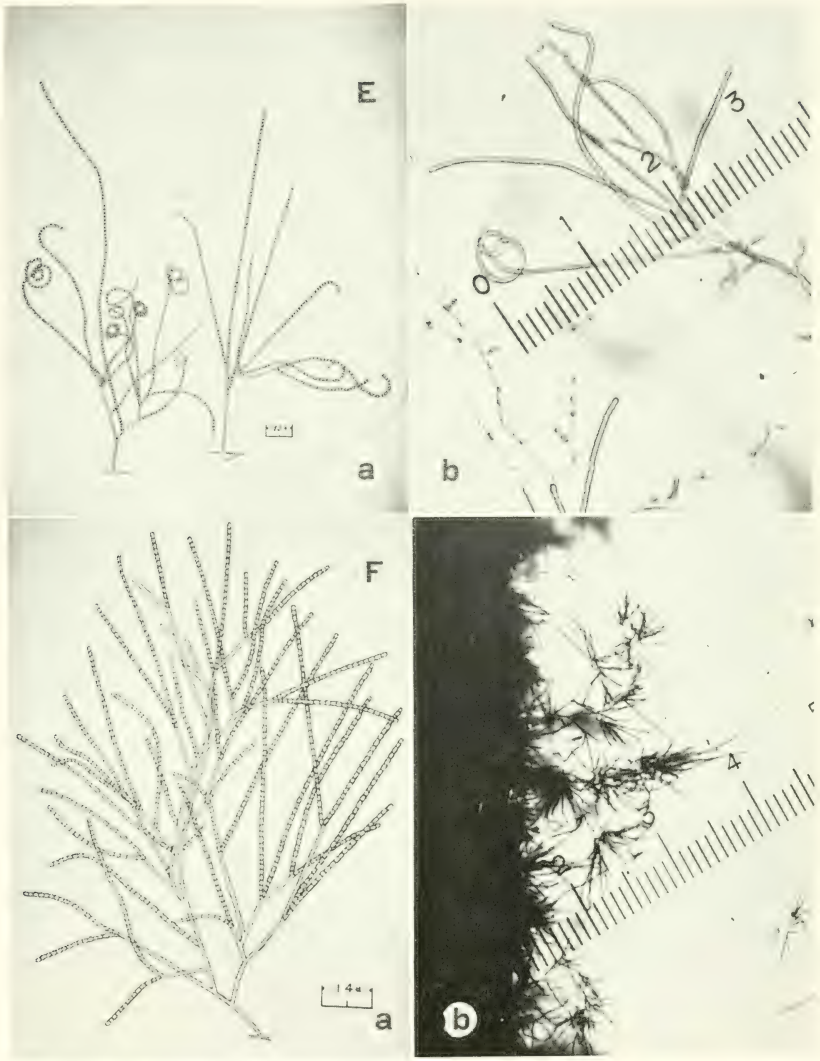

PLATE V. Morphological types, according to Nomi (1959). *a.* represents a schematic presentation of each type; *b.* gives the actual photograph.

E. Aerial hyphae in clusters, terminal filaments developing into sporophores, both spiral- and nonspiral-forming (*S. lavendulae, S. roseochromogenes*).

F. Aerial hyphae in clusters; no spirals or loops (*S. antibioticus*).

(*See continuation, next plate*).

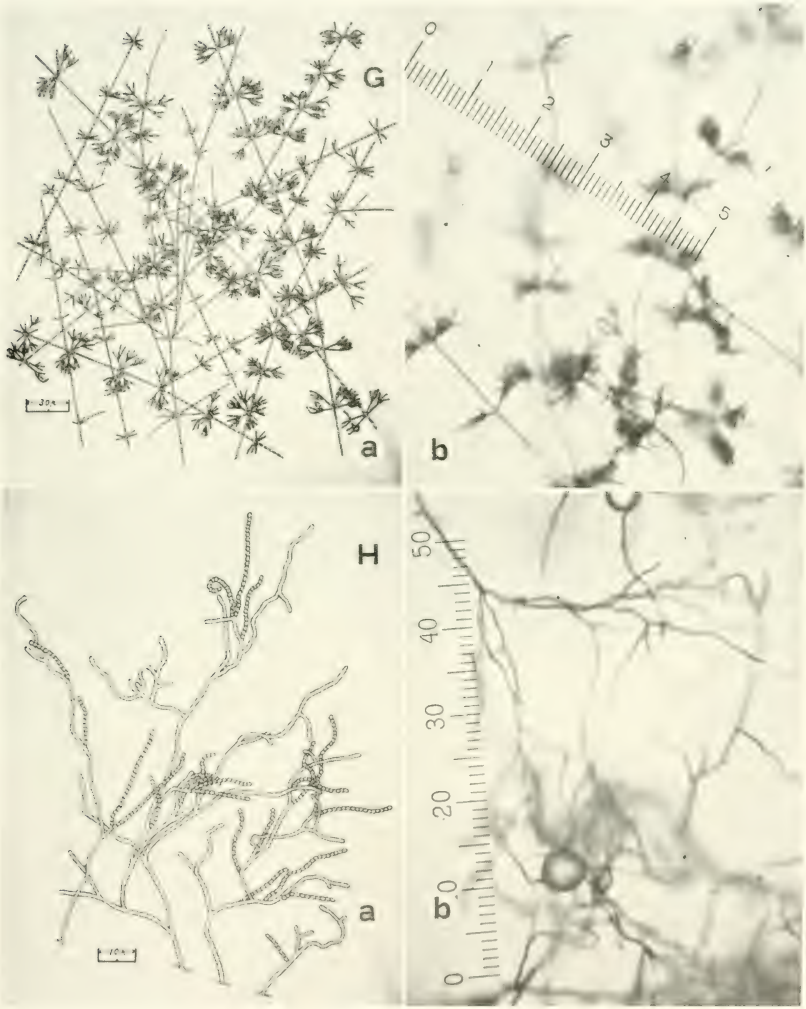

PLATE V. (*Continued*)

G. Aerial hyphae verticillate; no spirals (*S. hiroshimensis*).
H. Aerial hyphae flexuous or wavy; no long hyphae and no spirals (*S. albus*).

strate mycelium was taken as the basis for the primary subdivision into sections, and the color of the aerial mycelium for the secondary subdivision into series. In the systems used by Flaig and Kutzner (1954) and by Kutzner (1956), the color of the aerial mycelium was used in connection with that of the substrate mycelium. The system proposed by Yamaguchi and Saburi (1955) was based principally upon the structure of the sporophores, the color of the aerial mycelium being utilized in a secondary subdivision; in the final characterization, advantage was taken of the production of soluble pigments. A similar system was used by Shinobu (1958). Krassilnikov (1941, 1949), Hesseltine *et al.* (1954), Pridham *et al.* (1958), Ettlinger *et al.* (1958), Mayama (1959), and Nomi (1959) used morphological criteria for the primary subdivision of the genus.

Each one of the above systems has in itself certain serious limitations. It is necessary, therefore, to combine several properties in order to bring out the characteristics of the group or series, and especially those of the species.

Proposed System of Classification of the Genus Streptomyces *into Groups or Series*

In presenting the following system, full cognizance is taken of the criticisms to be directed against it, especially that the formation of the melanin pigment is given leading consideration, and that the production of other soluble pigments as well as of antibiotics is also given important consideration. I have felt that because of my own previous proposals, especially those incorporated in the various editions of Bergey's Manual, and my own interest in antibiotics, the best I could do would be to modify this system slightly. I hope that it will serve its purpose in the future as it has done in the past. The suggested series further broaden my earlier concept of species-groups. In view of the fact, however, that it is desirable for each group to be designated by a representative species, it has been found necessary, in some cases, to use a more recent, well defined species rather than one used long ago, for which no well established species is now recognized. This is true, for example, of the "chromogenes" series, which has been designated as *Phaeochromogenes*, for which a well recognized type culture is available.

I believe that the system of classification of the genus *Streptomyces* into series proposed here is simple and convenient. The use of ecological properties as a basis for the major subdivision of the genus, as in the last edition of Bergey's Manual, has been discarded. The thermophilic forms have been, for the most part, transferred to other genera (Chapter 11). The animal and plant isolates, including both pathogens and saprophytes, have been distributed throughout the genus, among the various series, where they logically belong on the basis of their morphological, cultural, and biochemical properties.

Both morphological (structure of sporophores) and cultural (color of aerial mycelium, melanin formation) characters are combined in the major subdivision of the genus into subgenera and into series. Each series is subdivided, on the basis of specific cultural and biochemical properties, into species. Formation of soluble pigments, pigmentation, and antibiotic production are also frequently taken advantage of in characterizing species. To identify a new culture properly, it is important to consider not only the series subdivision and species classification, but also the detailed description of each organism. Before it can be decided whether a newly isolated culture is different from one already described, a study should also be made of the varieties within the species previously created, as well as possible mutations and variations within the culture.

The names given for the various series are

the names of the type species within the particular series. (See also Table 10.)

Genus *Streptomyces* Waksman and Henrici, containing 16 series. Type species *S. albus* (Rossi-Doria) Waksman and Henrici.

A. Subgenus *Streptomyces* Waksman, with 14 series. Type species *Streptomyces* (*Streptomyces*) *albus* (Rossi-Doria) Waksman and Henrici.

B. Subgenus *Streptoverticillium* Baldacci, with 2 series. Type species *Streptomyces* (*Streptoverticillium*) *reticuli* (Waksman and Curtis) Waksman.

A. Sporophores straight, wavy, or spiral-forming. Subgenus *Streptomyces*

Subgroup A. MESOPHILIC

I. *Melanin-negative*

Series 1. *Albus*. This series is characterized by a white to light gray aerial mycelium, covering the whole of the substrate growth; concentric rings may be formed. It is melanin-negative. A faint brownish pigment may be produced on organic media. Sporophores are spiral-shaped, occasionally broom-shaped. The species within this series are usually strongly proteolytic, without formation of bad-smelling products. It may be argued that the type species *S. albus* is no longer available and that many species possess similar properties. This series and this species must be recognized historically, whatever the final decision of the type species to be adopted (Pridham and Lyons, 1960).

Series 2. *Cinereus*. This series is characterized by a gray aerial mycelium, ranging in color from light gray to mouse-gray or smoke-gray to ash-gray to bluish-gray; it may be white at first, later turning various shades of gray. Substrate growth may be colorless or yellowish, turning gray to dark. Frequently a soluble yellow pigment is produced. The sporophores are either straight or spiral-shaped.

Series 3. *Flavus*. This series, as well, has a long historical background; it was one of the three groups so designated by Sanfelice in 1904. It is characterized by a yellow or yellow-orange to yellowish-brown substrate growth, and by an aerial mycelium which is white to yellowish to gray. A yellowish-green to golden yellow soluble pigment is usually produced. Sporophores are long, straight, or spiral-shaped.

Series 4. *Ruber*. This series is characterized by a pink to orange to red substrate growth, and by a white to yellowish to red aerial mycelium. No soluble pigment is produced; occasionally a yellowish to brownish pigment may be formed. Sporophores are straight or spiral-shaped.

Series 5. *Viridis*. This series is characterized by a green to dark green substrate growth, and by a white to gray to light green aerial mycelium. Usually there is no soluble pigment; occasionally a light green pigment is formed. Sporophores are straight or spiral-shaped.

Series 6. *Violaceoruber*. Substrate growth is at first colorless, gradually becoming red or blue; aerial mycelium is white to gray with bluish tinge. The characteristic soluble pigment is blue, frequently changing in color with the reaction of the medium; it is blue at an alkaline and red at an acid reaction. Sporophores form spirals.

Series 7. *Fradiae*. This series is characterized by a yellow to orange substrate growth, and by a powdery pink to seashell pink to light orange aerial mycelium. Usually no soluble pigment forms on synthetic or organic media; a pink pigment may occasionally be produced. Sporophores are straight or spiral-shaped. Species are strongly proteolytic and antagonistic.

Series 8. *Griseus*. This series is characterized by colorless substrate growth, becoming, in certain media, brown to almost olive-black. Aerial mycelium is yellowish with a greenish tint, or greenish-gray or sea-green.

No soluble pigment is produced. Sporophores are straight or flexuous, producing tufts.

Series 9. *Hygroscopicus.* This series is characterized by a colorless substrate growth, which gradually becomes yellow, dark to almost black. Aerial mycelium is white to gray; it is often moist and even soft. Sporophores are straight and spiral-shaped. No soluble pigment is produced.

II. *Melanin-positive*

Series 10. *Scabies.* Aerial mycelium is white to gray to buff. Substrate growth is brown to black. Sporophores are straight or spiral-shaped.

Series 11. *Lavendulae.* Aerial mycelium is lavender to rose or pink to vinaceous lavender. Substrate growth is colorless to cream-colored. Sporophores are not flexuous, often forming loops and loose or open spirals.

Series 12. *Erythrochromogenes.* Aerial mycelium is white with brownish shade. Substrate growth is brown to black. Sporophores produce spirals.

Series 13. *Viridochromogenes.* Aerial mycelium is light green to olive-green. Substrate growth is grayish-green to brown to black. Sporophores produce spirals.

Subgroup B. THERMOPHILIC

Series 14. *Thermophilus.* This series comprises six species. These are listed in Chapter 11.

B. Sporophores produce verticils.
Subgenus *Streptoverticillium*
Melanin-negative

Series 15. *Cinnamomeus.* This and the next series are largely characterized by the morphological structure of their sporulating bodies. The sporophores produce verticils on the primary or on the secondary branches of the aerial mycelium, or on both. The spore chains are straight or spiral-shaped. This group is further characterized by being melanin-negative. The aerial mycelium is white to pinkish to cinnamon-colored.

Melanin-positive

Series 16. *Reticuli.* This series is characterized by the same morphological properties as Series 15, but it is melanin-positive. The aerial mycelium is white to gray.

There is a considerable overlapping of the different series. Frequently a given culture may be placed in one series or another, depending on the media and the conditions used for growing the organism, not to mention the idiosyncrasies of the observer. Classification becomes particularly difficult when one bears in mind the marked variations frequently observed between different isolates of the same species, and the tendency of individual cultures to mutate upon continued cultivation in artificial media. The fact that identification is frequently based upon comparison with published descriptions rather than with type cultures has resulted in the tendency to create new species on the basis of minor differences, some of which may be simple variations.

Most of the series are made up of non-chromogenic forms (or those that produce no melanoid pigments), although some of the constituent species may produce faint brown soluble pigments on certain media. Some of these pigments result from lysis of the mycelium of the organism; others may be quite distinct and chemically different from the typical melanoid or chromogenic pigments, *e.g.* the olive-green to olive-buff pigment frequently produced by *S. griseus.* Fewer series are composed of truly chromogenic forms, those capable of producing brown to dark brown or almost black soluble pigments with protein-containing media.

The various "series" suggested here are quite distinct from those proposed by Baldacci *et al.* (1954). They proposed, for ex-

ample, a "Bostroem" series, for which no true representative can be recognized at present. Their series "Antibioticus" and "Caeruleus" cannot be accepted for other reasons that need not be discussed further here. Certainly, the idea expressed by Baldacci *et al.* in 1955 that "it is not possible to speak of a natural systemization of these microorganisms at the present state of knowledge . . . for the time being, one must limit oneself to a classification aiming solely at diagnosis and nomenclature," represents a defeatist attitude. It is well illustrated by his creation of a series named "Diastaticus." Here were included pigmented and nonpigmented organisms, chromogenic and nonchromogenic, with such fantastic names as *A. rubrocyanodiastaticus*, and such varieties as *atrodiastaticus*. This is certainly a good cause for confusion.

Similar criticism can be applied to the grouping of the species proposed by Gause *et al.* (1957). Whereas Baldacci used the color of the substrate mycelium for primary subdivisions of the genus into sections, and the pigmentation of the aerial mycelium for the further division of the sections into series, Gause *et al.* (1957) omitted the sections altogether, and divided the genus directly into series largely on the basis of the pigmentation of the aerial mycelium. Descriptions of 37 old and 71 new species were reported by a group of six collaborators. The authorship was of a collective nature, with all the possibilities for confusing the credit to be assigned to each individual, since it is stated that "the study of the structure, classification, ecology and distribution of actinomycetes occupies the attention of large scientific collectives in a number of institutes and universities." Proceeding from the fact that so many new species have been recently created, in describing producers of antibiotics, these investigators assumed that this was further proof that the old systems of classification were insufficient. Although it was recognized that the pigmentation of the aerial mycelium, the major criterion for classification purposes, could change on continued incubation, as in the case of their Group I, "cultures with lavender and brownish-rose pigment may change in color to salmon, red, and pale terra cotta," nevertheless, 15 series were adopted. This fact alone would tend to cast doubt upon the significance of recognizing major groups solely on the basis of pigmentation of the aerial mycelium. In establishing the species, structure of the sporophores was used in some cases; in others, the pigmentation of a single medium, frequently unknown in composition, was used.* To complicate the situation further, authors of old species and emendations of species were incorrectly credited, providing a potential source of confused nomenclature.

Lieske (1921) was the classical "lumper," largely because of the limitations imposed by the use of complex organic media, and because he was not aware of some of the characteristic morphological and cultural properties of the organisms, brought out particularly on synthetic media. The "splitting" attitudes of Baldacci, Gause, and certain others have brought the system of classifying this important group of organisms to undesirable extremes.

In the decision to classify the genus *Streptomyces* into 16 series, it is well understood that in time other series will be added; some of those presented here may eventually be split into two or more series; some of the varieties may be raised to the status of species; or some of the species may be raised to the status of series.

The problem of whether antibiotic production is a species characteristic is still unsettled. Undoubtedly, the production of different antibiotics can be combined with

* Hottinger's, to which no reference is given.

certain other distinct properties, such as pigmentation, morphology, and carbon utilization, to justify the creation of new species. This has actually been done for the separation of *S. griseinus* from *S. griseus* and the raising of the latter to a series status. In other cases, however, the mere formation of a different antibiotic without other accompanying differences hardly justifies, for the present at least, the creation of new species.

Chapter 6

Series and Species of the Genus *Streptomyces*

The genus *Streptomyces* was created in 1943, to separate certain aerial mycelium-producing actinomycetes from the rest of the order Actinomycetales. Although there is considerable overlapping between species placed in this genus and those of *Nocardia* and some of the thermophilic groups, there are certain important properties that may be said to characterize this genus, thus separating it, if not for any other reason than that of convenience, from the others.

The major important characteristic properties that distinguish the genus *Streptomyces* from the others can be briefly summarized as follows:

1. A more or less branched, nonseptate, substrate or vegetative mycelium (stroma) is produced.

2. Growth takes place either on the surface of agar or gelatin media or penetrates deep into the medium, forming a compact, often leathery mass, designated as a colony.

3. During growth in stationary liquid media, no turbidity is produced except on lysis; the masses of growth appear as clumps or compact masses.

4. The surface colony gradually becomes covered with an aerial mycelium, though this occasionally may not occur.

5. The aerial mycelium produces sporogenous hyphae or fruiting bodies, which are straight, or in the form of tufts, or curved, spiral-shaped, or verticillate.

6. The sporophores carry chains of single-celled spores (or conidia), which vary in shape from spherical to oblong or cylindrical, and also in surface appearance when viewed with the electron microscope.

7. The vegetative growth, the aerial mycelium, and the spores *en masse* frequently are colored in a characteristic manner; the color may also dissolve into the medium, producing a "soluble pigment."

8. The species are aerobic and mesophilic, nonacid-fast and gram-positive.

The genus *Streptomyces* comprises, by far, the largest number of species of actinomycetes now known to occur in nature. The various species belonging to this genus differ greatly in their morphological, cultural, physiological, and biochemical properties. They include the majority of antibiotic-producing actinomycetes. The growing economic importance of these organisms has tended to increase the need for the separation of the genus into groups, each of which would contain one or more species. This need has recently been further emphasized by the creation of numerous additional species.

The color of the aerial and substrate mycelium, the morphology of sporophores, and the formation of melanin pigments have been largely used for the separation of the genus *Streptomyces* into series and species. For the supplementary characterization of

the species, the formation of nonmelanin pigments, the ecology of the organisms, and some of the biochemical properties (notably antibiotic formation) have been utilized. This is also true of their practical utilization for the production of enzymes, vitamins, or antibiotics.

It would appear that morphological properties might offer a natural and stable basis for a system of classification of these organisms. Unfortunately, certain characteristic morphological features of the genus *Streptomyces* undergo variation, depending upon the nutrition of the organisms and upon the environment. This tended to suggest, at first, the inadvisability of considering morphology as the major basis for the classification of the genus *Streptomyces*. This was true, for example, of Drechsler's idea of considering the type of curvature of the spiral-forming aerial hyphae as a basis for classification. It was also true of Waksman's suggestion that the mode of branching of the sporophores might be used for this purpose. The ideas of Krassilnikov (1941, 1949) in emphasizing the size and shape of the spore would also meet with similar criticism. Flaig *et al.* (1955), as well as Ettlinger *et al.* (1958), proposed use of the nature of the spore surface as a species characteristic; unfortunately, this property, depending as it does upon the use of the electron microscope, has not been readily enough established to enable the separation of the genus into groups and species.

Certain morphological properties are now, however, well recognized and can be utilized for the separation of certain groups of organisms belonging to the genus *Streptomyces*. Such groups possess sufficiently well defined morphological features to differentiate them from the rest of the genus. This is true particularly of those forms that produce radiating sporulating hyphae (verticils), with straight or spiral-shaped branches on the main sporophores or on the side branches. This property makes it possible to distinguish these particular forms from the majority of other species of *Streptomyces*, which produce either straight, flexuous, curved, or spiral-forming sporophores. Several systems of classification of the genus *Streptomyces* into series (Hesseltine *et al.*, 1954; Shinobu, 1958b) took full advantage of the verticil-producing property; Baldacci (1959) went so far as to suggest placing the latter into a separate genus. The separation of the spiral-forming from the straight sporophore-producing types into separate groups has also been frequently suggested.

In view of the above limitations, the only conclusion that can be reached is that, for the present at least, a logical system of separation of the genus *Streptomyces* into a number of distinct series should be based upon a combination of several of the morphological and physiological properties. It is proposed here to divide the genus into 16 series. This system, likewise, is open to criticism: (a) there is left, for example, considerable room for a certain amount of overlapping in some of the major properties which characterize the various series; (b) the position of a species within a series is not always well defined, and some of the species could frequently be placed with as much justification in one series as in another; (c) there is, further, a lack of uniformity in characterizing the various series: in some instances color of the aerial mycelium or of the substrate growth is used, and in others the formation of soluble pigments is emphasized.

Fully recognizing the above limitations, however, I feel that a sound basis has been laid, taking full advantage of the knowledge now available, for dividing the genus *Streptomyces* into series. As further information accumulates, the system can easily be modified, since it lends itself readily to various changes and modifications.

A detailed characterization of the various series is presented here. Some of the series are described in greater detail than others.

This is due either to their longer historical background or to their capacity to form important economic products, especially antibiotics.

In characterizing each series, the following properties have been given special consideration.

a. Morphological properties, notably structure of sporophores and spores.

b. Color of aerial mycelium on synthetic media.

c. Formation of soluble brown pigment (melanin) on protein media.

d. Spore surface.

e. Other characteristic properties, such as color of substrate growth, formation of soluble, nonmelanoid pigments, rate of proteolysis, or production of specific antibiotics.

A summary of the properties of the 15 series within the genus *Streptomyces* is given n Table 10.

I. Series *Albus*

Characteristic Properties

a. Sporophores produce spirals; spores spherical to oval.

b. Color of aerial mycelium white.

c. Melanin-negative.

d. No soluble pigment produced (except a faint brown pigment on certain media).

e. Weakly proteolytic and weakly antagonistic.

The *Albus* series comprises a large number of organisms, characterized by the production of a typical leathery and compact substrate growth, colorless on various media. Aerial mycelium is snow-white to white in color, assuming various shades as the culture grows older. The sporophores are long and form spirals; the spores are spherical to ovoid. The various strains grow well on both organic and synthetic media. They vary greatly in their proteolytic and diastatic properties. As a rule, this group of

TABLE 10

Characteristic properties of various series of Streptomyces

No. of series	Name of series	Melanin	Aerial mycelium	Color of growth	Spiral formation	Type species
1	*Albus*	−	White	Colorless	+	*S. albus*
2	*Cinereus*	−	White to gray	Colorless to yellow	+−	*S. craterifer*
3	*Flavus*	−	Mouse-gray	Yellow	+	*S. flavus*
4	*Ruber*	−	Rose	Red	+−	*S. ruber*
5	*Viridis*	−	Gray to green	Green	+−	*S. viridis*
6	*Violaceoruber*	−	Gray	Red to blue	+	*S. violaceoruber*
7	*Fradiae*	−	Pink to rose	Yellow to orange	+−	*S. fradiae*
8	*Griseus*	−	Grass-green	Colorless to olive-buff	−	*S. griseus*
9	*Hygroscopicus*	−	White to gray	Dark gray to black	+	*S. hygroscopicus*
10	*Scabies*	+	Gray	Brown to black	+	*S. scabies*
11	*Lavendulae*	+	Lavender	Colorless	+−	*S. lavendulae*
12	*Erythrochromogenes*	+	Yellowish	Orange	+−	*S. erythrochromogenes*
13	*Viridochromogenes*	+	Green to olive-green	Brownish to green	+	*S. viridochromogenes*
14	*Cinnamomeus*	−	Pinkish	Yellowish	−	*S. cinnamomeus*
15	*Reticuli*	+	White to gray	Colorless	+−	*S. reticuli*

organisms, so far, has not been reported as containing any significant antibiotic-producing forms. Although one of the first preparations possessing certain antibacterial properties ever recorded for a culture of an actinomycete was said to have been obtained from a member of the *Albus* group (Gratia and Dath, 1925), it is open to question whether the particular culture was a true *S. albus*. According to Pridham and Lyons (1960), this organism should be considered as more closely related to the *Griseus* group.

Species belonging to the *Albus* series are found in soil and in dust. Various early investigators, notably Almquist, Gasperini, Rossi-Doria, Beijerinck, and Sanfelice, reported the isolation of organisms belonging to this series.

Various systems of classification of the *Albus* series have been proposed. Attention may be directed here to the fact that at one time or another all the sporulating actinomycetes, especially the saprophytic forms, mostly now recognized as belonging to the genus *Streptomyces*, were classified (see Beijerinck, 1900, for example) into two groups: (1) *A. albus* (*Streptothrix alba*), comprising those forms that produce a white aerial mycelium and no soluble pigment; (2) *A. chromogenus* (*Streptothrix chromogena*), including those forms that produce a black pigment on protein media. Duché appeared to follow this system as late as 1934, since he included in his monograph (Duché, 1934) on the actinomycetes only those species that were said to belong to the *A. albus* group. In view of the significance of the specific name "*albus*," representing the type culture of the genus *Streptomyces*, it may be of interest to trace the usage of this name in the literature on the actinomycetes.

In presenting this historical summary, the writer has taken full advantage of the comments concerning this group made by Baldacci (1939), who is frequently quoted here almost verbatim. Baldacci recorded about 30 synonyms, some of which are listed in Table 11. (Others were not included, since they are not considered as typical of the group).

The name "*alba*" was first applied to an actinomycete culture by Rossi-Doria (1891). He established the characteristics of this organism, indicating its synonymy with the cultures previously characterized by Almquist (1890); he also identified it with a culture designated as *Streptothrix Foersteri*, isolated from the air by Gasperini (1890). Rossi-Doria refused to accept the identification of this organism with *Streptothrix Foersteri* Cohn. He said: "Nothing in the description given by Cohn can justify such an idea. In that description, in fact, only generic characters are given; of specific characters there does not exist even a shadow." The three cultures of Almquist appeared to differ little among themselves. Since one (culture I) was said to form a white crust changing in time to gray, Baldacci preferred to exclude it from the synonymity with the *A. albus*. Gasperini (1894) recognized the difference between *A. albus* and *A. chromogenus*. The latter possessed chromogenic properties, the pigment diffusing into the substrate.

Sanfelice (1904) was the first to divide into three groups the actinomycetes now recognized as belonging to the genus *Streptomyces*, using *S. albus* as the representative of the first of these groups. He noted that some of the cultures belonging to this group may produce a black pigment when grown on potato. He added quite significantly: "On the basis of this observation, a superficial observer may create a new species out of a pigmented culture without considering the fact that it originated from *Str. alba*."

Krainsky (1914) isolated from garden soil a culture which he described as *A. albus*. This culture produced a well developed growth, white at first, then becoming gray on certain media such as glucose agar and

TABLE 11

Comparative characters of Streptomyces albus *and related species* (Baldacci, 1939)

Specific name	Author	Nature and dimensions of spore	Mycelium		Chromogenesis	Proteolytic action	
			Substrate	Aerial		Gelatin	Milk
		μ					
Cladothrix dichotoma	Macé	+		White	+		
Streptothrix Foersteri	Gasperini	Oval (1.0–1.5)	Colorless to yellowish	White	−	+	
Streptothrix n. 2	Almquist	+		White	−	+	
Streptothrix n. 3	Almquist	+		White	−	−	
Streptothrix alba	Rossi-Doria	+	Colorless to black	White	−		−
Actinomyces albus	Krainsky	Oval (1.0)	Colorless	White (less often gray)	−	+	
A. albus	Waksman and Curtis	Spherical, oval (1.2–1.6 by 1.1–1.4)	White or gray	Cream or gray	−	+	+
A. albus	Jensen	Rectangular (0.4–0.5 by 2.4)	Cream or yellow-ocher	White	+		
A. albus	Duché	+	White-yellow	White	−		−
A. chromogenus	Gasperini	Oval (1.5)	Ocher to black	White	+		
Cladothrix odorifera	Rullmann	+ (1.0)		White or chalk white	Weak	+	
A. thermophilus	Berestnew	+		White			
A. thermophilus	Gilbert	+ (0.5–0.6)	Gray-yellow	Gray		+	+
A. thermodiastaticus	Bergey	Oval	Colorless	White		+	−
A. sanninii	Ciferri	Round	Colorless	White or ivory white	+	Weak	
A. almquisti	Duché	+	Yellowish	−	−		
A. gougeroti	Duché	+	Greenish	White	−		
Streptothrix gedanensis I	Scheele and Petruschky	+	Colorless	White		Weak	
Streptothrix candida	Petruschky	+	Colorless	White		+	+
Streptothrix lathridii	Petruschky	+	Colorless	White		+	+
Cladothrix invulnerabilis	Acosta and G. Rossi	+		White	−	+	

gelatin. The aerial mycelium was produced readily, the medium remaining colorless. The spores were oval, 1 μ in size. Gelatin was liquefied. Nitrate was reduced. The culture had no diastatic action on starch.

Waksman and Curtis (1916) isolated from soil cultures of an organism considered to be *A. albus*. It was similar to that of Krainsky, although the exact identity of the two was doubted. The diagnosis of Krainsky was, therefore, amended. The aerial mycelium appeared either white or gray, according to the composition of the medium; the substrate growth varied from white to gray. The sporophores produced short and rare spirals. The spores were 1.2 to 1.6 by 1.1 to 1.4 μ. The culture was nonchromogenic, hydrolyzed starch, reduced nitrate, and liquefied gelatin. Jensen (1931) also isolated a culture of *A. albus* from soil. The aerial mycelium was constantly white, and the substrate growth cream-colored or yellow-ochre. The culture was said to produce cylindrical spores, 0.4 to 0.5 by 2.4 μ.

Duché (1934) created an excessively broad "*A. albus* group." He was severely criticized by Baldacci (1939), who said that "if it is meant by 'albus group' those species that produce white aerial mycelium in culture, they are many more in numbers than those described by Duché. These are also the 'viridis' species and those that show analogy with the 'flavus' forms." Baldacci further emphasized that *A. albus* is a well characterized species that does not permit confusion with others, while the "albus group" of Duché comprised 18 species, of which 15 were new ones; among these were

such forms as *A. viridis*, *A. albidoflavus*, and *A. alboflavus*, which definitely belong to other groups.

In his morphological study of the actinomycetes, Duché recognized five types of sporulation, none of which was used for systematic purposes. The relationship of aerial hyphae, spirals, and spores was not sufficiently emphasized. Duché stated that colonies may also originate from arthrospores, implying thereby that any mycelial fragments will reproduce and multiply in the culture. He spoke, however, of "monosporic" colonies, although he used the method of successive dilutions and not that of single-spore isolation. He documented the various interpretations of the species *A. albus*, stating at first that the description of Waksman "ne correspond pas tout à fait aux type albus de Gasperini, Rossi-Doria et Krainsky." On comparing his own culture with the preceding ones, he stated, "L'espèce de Waksman and Curtis semble posséder toutes les propriétés de celle de Krainsky. . . . Notre espèce resemble aux deux précédentes." Baldacci concluded that the work of Duché, after trying to prove the diversity of the various interpretations in the literature, had not attained its purpose of establishing what the species *A. albus* should be.

In proposing the genus *Streptomyces* in 1943, Waksman and Henrici stated: "We have selected as the type species of this newly named genus, *Streptomyces albus* (Rossi-Doria *emend.* Krainsky) comb. nov. This species was formerly known as *Actinomyces albus* Krainsky and first described as *Streptothrix alba* Rossi-Doria. This is one of the commonest and best known species of the group, and, although it may later be subdivided into further species, it is at present as definite as any other. It has been recently studied intensively by Duché (1934) and by Baldacci (1939). It is colorless, with white aerial mycelium, forming ovoidal spores in coiled chains on lateral branches of the aerial hyphae. It is proteolytic, liquefying gelatin and peptonizing milk with the production of an alkaline reaction in the latter. It does not produce any soluble pigment either on an organic or synthetic medium, but does produce a characteristic earthy or musty odor."

Pridham and Lyons (1960) have recently made a comprehensive analysis of the present status of *Streptomyces albus*. Their study was based upon a detailed examination of 55 cultures collected from various sources. They came to the conclusion that "there has existed since about 1916, two entirely different concepts with regard to the nature of *Actinomyces* (*Streptomyces*) *albus*. One concept centers around strains with the following characteristics: flexuous fruiting bodies, colors of aerial mycelium in tints and shades of olive-buff (yellowish-gray or tan); nonchromogenicity (inability to form brown, deep brown, or black diffusible pigments in organic substrates); and marked abundance in nature" (these strains are now considered as comprising members of the *Griseus* group). "The other concept concerns strains that are characterized by coiled or spiralled fruiting bodies with catenulate ovoidal spores; by aerial mycelium colors generally interpreted as cretaceus (chalk-white, often with faint tinges of pink); by nonchromogenicity (inability to form brown, deep brown, or black diffusible pigments in organic substrata); and by their relative rareness in nature" (these strains are now considered as *Albus* group proper).

Morphologic Characters

Various methods were used in the study of the morphology of *S. albus*. Baldacci observed two types of mycelium. One was hyaline, not less than 1 μ in diameter, ramifying more or less abundantly, and having an undulated appearance. The ramification starts perpendicularly from the point of

intersection, but it can follow in other directions or assume a wavy appearance. This mycelium originates directly from the germinating spores and can be rather abundant. It corresponds to the first vegetative growth and is designated as "substrate mycelium." The second type of mycelium is more distinctly visible than the first. It is larger in diameter (1.1 to 1.4 μ); it is subhyaline with a tendency to assume yellowish coloration. This mycelium carries abundant sporogenous hyphae, scarcely ramified. The extremities curve to hook shapes and successively turn to spirals. This mycelium is white and is superimposed on the substrate growth; in time, it turns to dirty white or milky white, powdery or crusty. It is designated as "aerial mycelium."

The branches of the aerial mycelium become sporophores and give rise to spores that are formed by contraction. The spores are short, oval in shape, white, and not without a certain polymorphism, appearing sometimes as short rods. One may also observe round forms, but they must be interpreted as spores seen in a vertical projection. The spore dimensions are 0.6 to 0.7 by 1.2 μ. According to Baldacci, the spores are smaller than those observed by Jensen and longer than those described by Drechsler. Baldacci was not sure, however, that Drechsler's culture corresponded to *S. albus*. The difficulty in reaching an agreement concerning the shape and measurement of the spores is not due, as claimed by Duché, to their small size, but to the time at which the measurements are taken and observations made. When the spores are united in chains in the sporophores, they appear longer and rectangular; if measured when they are spread in the preparation, they appear shorter. It is natural, therefore, that only the shape and dimension in the latter case be accepted. It should be noted that the free spores are not distributed on the slide in a uniform manner, because many remain attached to the glass at their smaller surface, and thus appear round. Spirals are abundant.

The degeneration of the hyphae can be observed in the old substrate mycelium: zones of protoplasm with empty spaces simulating arthrospores can be seen. This phenomenon led earlier investigators, including Gasperini, to make unjustified generalizations. This is also evident in the claims of Lachner-Sandoval, Vuillemin, and Grigorakis, who observed this false sporulation. Although these authors did not always specify the particular species used in their studies, Baldacci was inclined to think that they had to do rather with a *Nocardia*; the vegetative mycelium of the latter, when its growth is arrested, subdivides into fragments that look like bacillary elements. This type of fragmentation was studied by Ørskov and by Jensen. The "arthrospores irregulars" of Duché and others bring out very clearly this type of degeneration, which was erroneously interpreted as a type of sporulation.

Cultural Characters

S. albus produces a colony in the form of a growth adherent to the substrate; it is wrinkled and colorless. The aerial mycelium appears first in the drier portion of the colony; it is chalk-white in color, as if lime had been sprayed over it. On aging, it turns to ivory-white; on some media, such as nutrient agar, it assumes a grayish tint. With age, growth of the culture becomes opaque or even yellowish, comparable to the color of the substrate, as can be observed in the cultures where the aerial mycelium is not produced. The formation of the aerial mycelium appears to correspond to the presence of water of condensation, the aeration of the cultures, and various other factors, independent of the strain or variety. The aerial mycelium is more or less abundant and may cover the entire colony.

Concentric ring formation may be observed in smaller colonies, as described by Rossi-Doria. The cultures produce the characteristic moldy or soil odor.

On synthetic agar, the initial development of the culture is characterized by a dusty or powdery white, dry growth, forming furrows or concentric rings. The substrate mycelium is formed in a thin, scarcely visible layer over the agar. The colony does not assume a vigorous aspect. The white aerial mycelium appears late. Pigmentation of the agar is seldom observed, except for a few strains that show feeble chromogenesis in this medium.

On potato, the development is rapid, with small, partly confluent colonies or in the form of extended membranous growth that becomes covered with white aerial mycelium.

Biochemical Properties

Temperature: Optimum 24–28° (24–44°)C.

Gelatin: Liquefied.

Starch: Diastatic action variable.

Sucrose: Inverted.

Nitrate: Reduced to nitrite.

Antagonistic properties: The organisms belonging to the *S. albus* group are usually weak antagonists. Some cultures possess activity against gram-positive bacteria.

Species

Krassilnikov (1949) included 19 species in the *Albus* series. He used a combination of different criteria for their separation and identification. For separation of the cultures, he considered the odor produced as the major criterion, which is rather unreliable. Use of this criterion is largely responsible for the inclusion, in this group of species, of forms designated as *aromaticus*, *odoratus*, *odorifer*, *putrificus*, etc. Krassilnikov also considered, for identification purposes, the shades of white in the aerial mycelium, temperature relation, proteolytic and antagonistic properties, the secretion of a brown substance, growth in acid media, production of ammonia and H_2S, decomposition of rubber, and formation of coremia. He included in this group various thermophilic and thermotolerant organisms.

Gause *et al.* (1957) divided the *Albus* series, on the basis of the color produced on a complex organic medium, into three subgroups, comprising five species and one variety:

a. Medium not pigmented: *A. candidus*, *A. candidus* var. *alboroseus*, and *A. albidoflavus*.

b. Medium colored brown: *A. longisporus* and *A. mirabilis*.

c. Medium colored brownish: *A. alborubidus*.

The above characterization fails to recognize some of the fundamental cultural properties of actinomycetes, namely, the production of melanin pigments in protein media and the structure of the sporophores. The resulting subdivision of such a group into subgroups thus loses all significance. Only one of the above species (*A. albidoflavus*) is found in Krassilnikov's "albus" series. Kutzner (1956) reported that he had obtained four strains of *S. albus* from different institutions and found them to be identical.

Baldacci placed in the *Albus* series the following organisms, either because they were considered as synonyms or because they were believed to be closely related: *Actinomyces albus*, *A. acidophilus*, *A. almquisti*, *A. beddardi*, *A. chromogenus*, *A. erythreus*, *A. exfoliatus*, *A. farcinicus*, *A. flocculus*, *A. gedanensis*, *A. gelaticus*, *A. gougeroti*, *A. heimii*, *A. kimberi*, *A. lieskei*, *A. listerii*, *A. malenconi*, *A. reticuli*, *A. somaliensis*, *A. sanninii*, *A. saprophyticus*, *A. thermophilus*, *A. upcottii*, *A. willmorei; Cladothrix dichotoma*, *C. liquefaciens*, *C. invulnerabilis*, *C. odorifera; Oospora doriae*, *O. alpha; Streptothrix alba*, *Str. candida*, *Str.*

dassonvillei, *Str. foersteri*, *Str. graminearum*, *Str. leucea*, *Str. lathridii*, and *Str. pyogenes*. Such a large conglomeration defeats completely the purpose of grouping, since the above forms vary greatly morphologically, culturally, and physiologically.

A number of species can, however, be included in this series. It is sufficient to mention *S. albus*, *S. calvus*, and *S. niveus*.

II. Series *Cinereus*

Characteristic Properties

a. Sporophores straight or spiral-shaped.

b. Color of aerial mycelium white to gray; occasionally dark humid stains or guttation drops.

c. Growth usually colorless, occasionally yellow to tan.

d. Melanin-negative.

When members of this series form an aerial mycelium, the color is characteristically gray. Although it may be white at first, it changes to various shades of gray, ranging from light gray to mouse-gray to bluish-gray or even vinaceous-gray or blackish-gray. Frequently white spots are produced in the aerial mycelium. The substrate growth is often colorless or gray, occasionally becoming yellowish to buff-colored; it is either opaque or somewhat slimy; the reverse is usually colorless, occasionally turning yellow to tan. It is melanin-negative. Occasionally a yellowish or brownish soluble pigment may be produced. The sporophores are straight, often formed in clusters or tufts; they may also produce spirals that are either open or compact.

This series is widely distributed in nature. It comprises, in addition to well described species given here, a great number of incompletely described forms, as shown in Chapter 12.

A variety of antibiotics have been found to be produced by members of this series.

Some Characteristic Species

The following species may be tentatively included in this series: *S. craterifer*, *S. intermedius*, *S. parvullus*, and *S. cellulosae*.

One may also include in this series various organisms described by Krassilnikov (1949), including his emendation of *A. griseus* Krainsky (not *S. griseus* Waksman), *A. griseus variabilis*, and *A. griseus zonatus*. This is also true of some of the forms described by Gause *et al.* (1957) under such names as *A. rubiginosus*, *A. griseomycini*, *A. iverini*, *A. acrimycini*, *A. acrimycini* var. *globosus*, *A. atroolivaceus*, and *A. griseorubens*.

III. Series *Flavus*

Characteristic Properties

a. Sporophores long, spiral-shaped; spores spherical to oval.

b. Aerial mycelium white to gray to mouse-gray; color of growth yellow to golden yellow.

c. Melanin-negative.

d. Yellowish-green to golden soluble pigment may be excreted into the medium.

e. Strongly proteolytic and antagonistic.

The *Flavus* series includes a large number of organisms, widely distributed in nature. The members of the series vary greatly in some of their cultural, biochemical, and morphological properties when grown on artificial media. This group has been recognized since 1891, when Rossi-Doria described a culture under the name of *Streptothrix albido-flava*. Another similar culture was soon described by Gasperini (1892) as *Actinomyces albido-flavus*. Sanfelice (1904) designated the second of his three groups as *Str. flava*, comprising organisms isolated from the air. Caminiti (1907) was inclined to include in this group various pigmented forms, such as *Str. citrea* and *Str. chromogena*.

Numerous organisms belonging to the

Flavus series have been isolated from soil, dust, and other natural substrates by Krainsky, Waksman and Curtis, and others. They have been designated by a variety of names, such as *S. alboflavus*, *S. aureus*, *S. citreus*, *S. griseoflavus*, and *S. flaveolus*.

The *Flavus* series is characterized by cream-colored to yellow or golden yellow growth on most artificial media. The aerial mycelium is usually white to gray to mouse-gray. The sporophores are long, usually spiral-shaped. The spores are spherical, usually 0.7 μ in diameter. No brown pigment is produced on protein media. A yellowish-green to golden pigment is often formed in synthetic and organic media. The various species in this group are strongly proteolytic and diastatic. Sucrose is inverted. Nitrate is reduced. Many of the strains are strongly antagonistic and are able to form active antibiotics, some of which have found extensive application as chemotherapeutic agents.

Krassilnikov (1949) recognized 13 distinct species as belonging to the *Flavus* series. Baldacci (1939), however, subdivided the actinomycetes with the characteristics of the *Flavus* series (various species producing yellow or golden growth) into a number of series: "*aureus*," "*albidoflavus*," "*sulphureus*," "*antibioticus*," and "*hygroscopicus*." Baldacci *et al.* (1954) included in the "*Aureus*" series such species as *S. aureus*, *S. aureofaciens*, *S. citreus*, *S. fimicarius*, *S. flavus*, *S. flaveolus*, *S. fordii*, *S. griseoflavus*, *S. hygroscopicus*, *S. microflavus*, and *S. parvus*.

A culture of an organism isolated by Takahashi (1953) in Japan was identified by him as *S. flaveolus* Waksman. To validate this identification, his description of this culture is presented in Table 12 alongside Waksman's description of the original type culture. These data show that, in spite of minor variations in color characterization, quantitative differences in gelatin liquefaction and nitrate reduction, and even in differences in antibiotic production, the identification of the species appears to be correct.

The same is true of the characterization of *S. parvus*. The original culture of this organism, which was used as the basis for its description in Bergey's Manual, has died out in the collection. Benedict, of the Northern Regional Research Laboratory of the U. S. Department of Agriculture, isolated from a sample of soil collected in West Africa a culture which he identified as *S. parvus*. A comparison was made of the culture originally isolated and described by Krainsky (1914), the culture isolated by Waksman and Curtis (1916) and reported in Bergey's Manual, and the new culture of Benedict (Table 13). The results point definitely to the identity of the three cultures, thus proving again that accurate identification of some species can be made by comparing freshly isolated cultures with written descriptions of type isolates.

Finally, a comparison was made (Waksman, 1957) of two published descriptions of *S. flavus* and *S. griseoflavus*, together with recent descriptions of two cultures that have been raised to the status of new species, namely, *S. aureofaciens* and *S. rimosus*, both important producers of antibiotics. The results, presented in Table 14, show that *S. aureofaciens* and *S. rimosus* are sufficiently different from *S. flavus* and *S. griseoflavus* to warrant the creation of new species. *S. aureofaciens* is characterized by a deep gray aerial mycelium, by a lack of or limited spiral formation, by limited proteolytic activity upon gelatin and milk, and by poor growth on nutrient agar. It was concluded that these properties differentiated this culture sharply from the two older cultures. *S. rimosus* is characterized by poor growth on synthetic agar and by the formation of abundant spirals in its aerial mycelium. These properties, together with certain

TABLE 12

Identification of Streptomyces flaveolus (Waksman, 1919; Takahashi, 1953; Waksman, 1957)

Characteristics	Waksman	Takahashi
Morphology		
Sporophores	Numerous spirals on all media	Numerous spirals on synthetic media
Spores	Oval to elliptical	Spherical or oval, 0.8 by 1.2 μ
Synthetic agar		
Substrate growth	Light sulfur-yellow turning cadmium-yellow	Antimony-yellow to chamois-colored
Aerial mycelium	White with ash-gray patches	White, later smoke-gray
Soluble pigment	Empire-yellow	Buff-yellow
Calcium malate-NH_4Cl agar		
Substrate growth	Cream-colored	Pale olive-buff to yellow-ocher
Aerial mycelium	Mouse-gray, with white margin	Vinaceous-buff to light mouse-gray
Soluble pigment	None	None or faint yellowish
Nutrient agar		
Substrate growth	Wrinkled, white	Colorless to whitish, reverse cinnamon-buff
Aerial mycelium	Abundant, white	White
Soluble pigment	None	Golden yellow
Gelatin		
Substrate growth	Abundant yellowish pellicle	Wrinkled, yellow
Aerial mycelium	White	White
Soluble pigment	Golden to faint brown	Faint yellowish-brown
Liquefaction	Rapid	Medium
Potato		
Substrate growth	Wrinkled, cream-colored	Wrinkled, golden yellow to orange
Aerial mycelium	White	White to seashell-pink
Color of plug	Faint brown	Faint brownish
Glucose broth		
Substrate growth	Thin, yellow pellicle	Colonial buff to honey-yellow
Aerial mycelium	White	White to smoke-gray
Soluble pigment	Golden	Yellowish (golden yellow)
Milk	Rapid coagulation and peptonization	Rapid coagulation and strong peptonization
Nitrate reduction	Strong	Positive
Antibiotic production	Produces actinomycin	Produces flaveolin

other morphological and cultural differences between this culture and the two older cultures, justified creation of a separate species, especially because of the ability of *S. rimosus* to produce an important new antibiotic.

In view of the great variability of these organisms and the temptation to establish separate species on the basis of minor differences in pigmentation, any attempt to create such new species must be considered critically.

A culture described as *S. armillatus* (Maney-Courtillet *et al.*, 1954) appeared, on the basis of the description, to be sufficiently close to *S. rimosus* to throw doubt upon its distinct identity. Like the latter, it produced spirals in its aerial mycelium; on synthetic agar it formed very poor growth without any aerial mycelium and without pigmentation; on nutrient agar, it produced yellow-gray growth with poorly developed white aerial mycelium and no soluble pigment; on potato

TABLE 13

Characterization of Streptomyces parvus (Waksman, 1957)

Characteristics	Krainsky	Bergey's 6th Edition	New culture received from N. R. R. L No. 3686
Substrate growth		Golden yellow to brick-red depending on composition of medium	Bright yellow
Aerial mycelium	White to gray to rose-yellow depending on nitrogen source	Poorly developed, rose-white; sporophores produce spirals	Long, straight hyphae; no spirals
Spores	More or less oval, 1.6 μ in size	Spherical to oval, 0.9–1.3 by 1.2–1.8 μ	Short, oval
Synthetic agar	Colonies small, yellow in color, with light colored aerial mycelium*	Colonies small, yellow, with aerial mycelium light yellow	Thin, yellow growth; thin white to yellow aerial mycelium; bright yellow soluble pigment
Nutrient agar	Yellow growth. Aerial mycelium appears late		Yellow growth; abundant white with grayish tinged aerial mycelium; bright yellow soluble pigment
Glucose asparagine agar			Yellow growth; white to gray aerial mycelium, golden soluble pigment
Glucose agar	Aerial mycelium light yellow; appears late	Colonies small, yellow, with aerial mycelium light yellow	
Gelatin	Colonies flat or concave, yellow in color; gelatin slowly liquefied	Colonies yellow; liquefaction medium	Cream-colored growth dropping to bottom; good liquefaction; bright yellow soluble pigment
Potato	Colonies yellow, aerial mycelium white		Abundant wrinkled brownish-yellow growth; abundant sulfur-yellow aerial mycelium; no soluble pigment
Cellulose	White surface growth	Growth good	
Remarks	Produces diastase; reduces nitrate slowly; strongly proteolytic	Produces actinomycin; reduces nitrate slightly	

* Calcium malate agar

plug, it produced yellow-gray growth with a faint brownish soluble pigment; on gelatin, it formed a surface growth with white aerial mycelium, with a yellowish or brownish soluble pigment, and good liquefaction of the gelatin; on milk, it produced good grayish growth. These characteristics, together with the ability to produce oxytetracycline, definitely placed the culture in the *S. rimosus* species. Emphasis was laid upon the fact it formed flat colonies, hardly folded and not cracked like those of *S. rimosus;* it showed concentric circles in the aerial mycelium, a variable property. It did not form nitrite from nitrate, and it did not hydrolyze starch; these two properties were hardly sufficient, however, to justify the recognition of *S. armillatus* as a new species.

Among the various members of the *Flavus* series, the actinomycin-producers occupy an

TABLE 14

Characteristics of Streptomyces flavus *and allied strains* (Waksman, 1957)

Characteristics	*S. flavus* (Bergey)	*S. griseoflavus* (Bergey)	*S. aureofaciens* (Duggar)	*S. rimosus* (Sobin *et al.*)*
Morphology				
Sporophores	Straight, much branched, no spirals	Straight, no spirals	Straight, flexuous; no spirals as a rule; occasional loose spirals	Numerous spirals
Spores	Oval		Spherical to oval, 1.5 μ long	Short, cylindrical, 0.6–0.7 by 0.8–1.4 μ
Synthetic agar				
Substrate growth	Yellow to sulfur-yellow	Reddish-brown to orange	Heavy cream-colored, becoming yellowish-brown.	Submerged, colorless
Aerial mycelium	Straw-yellow	White	White, turning mouse-gray to brownish-gray	None
Nutrient agar				
Substrate growth	Cream-colored, lichenoid	Cream-colored	Good, light brownish	Cream-colored to brownish
Aerial mycelium	White to light gray	White	None	None or white to gray-white
Soluble pigment	None	None	None	Faint yellowish or none
Gelatin				
Substrate growth	Yellowish	Cream-colored to brownish-white		Moderate
Aerial mycelium	None	White		White
Liquefaction	Positive	Slow	None	Medium to good
Soluble pigment	Faint yellowish	Faint yellowish		
Potato				
Substrate growth	Lichenoid, brownish to greenish-olive	Lichenoid, brownish to reddish-brown	Wrinkled, orange-yellow	Wrinkled, ochroid
Aerial mycelium	White to gray	White to gray		Whitish to drab
Color of plug	Brownish or none	None	Unchanged	Yellowish-brown pigment or none
Yeast-glucose agar				
Substrate growth	Rapid, lichenoid, brownish	Cream-colored to brownish	Heavy, cream-colored	Good, yellowish
Aerial mycelium	White, later grayish	White to grayish	White to deep gray or dark gray	Pallid drab
Soluble pigment	Yellow	Yellowish	None	Yellowish
Milk	Coagulation and peptonization	No coagulation, rapid peptonization	No coagulation, no peptonization	No peptonization
Production of antibiotics	An antibacterial agent formed		Chlortetracycline	Oxytetracycline and rimocidin

* An earlier description of this culture on synthetic agar was incorrectly labelled; the medium was made up at that time with glucose in place of sucrose, which explains the difference between the previous and present observations.

interesting place. A culture (No. 3491) belonging to *S. flavus* or to *S. parvus* was isolated in our laboratories in 1948 and found capable of producing actinomycin. It was nonchromogenic and formed a straw-colored to yellow aerial mycelium. This culture was found to belong to the *S. flavus* subgroup; other cultures (Nos. 3677, 3679, and 3680) were included in the *S. parvus* subgroup. Another culture (No. 3686), designated as *S. parvus*, did not form any spirals. Still another culture (No. 3687) produced only limited curling of the aerial mycelium and might be considered an intermediate between the two subgroups. It was suggested, therefore (Waksman and Gregory, 1954),

FIGURE 31. Variation in morphology of spore-bearing hyphae in *S. aureofaciens*: (left) natural variant A 377; (center) natural variant AB 374; (right) induced mutant A 377–2655 (Reproduced from: Backus, E. J. *et al.* Ann. N. Y. Acad. Sci. **60:** 101, 1954).

that the whole series be designated as *Flavus-parvus*. Considerable variation was found among the members of this series.

Morphological Characters

Hyphae: (a) short, gnarled, and in clusters, with short oval spores; or (b) long, straight with spherical spores; or (c) long with long corkscrew spirals and spherical spores (Fig. 31).

Physiological Characters

Sucrose nitrate agar: Growth cream- colored, yellow to brownish to orange; reverse yellow to orange. Aerial mycelium cream-colored, straw-colored to citron-yellow, straw-yellow, grayish-yellow to bluish-gray to white, or absent. Soluble pigment light yellow to brownish.

Glucose-asparagine agar: White to cream-colored growth, sometimes turning orange. Aerial mycelium white to gray. Soluble pigment none, or brownish to yellow.

Nutrient agar: Growth cream-colored to yellowish to brownish. Aerial mycelium white, cream-colored to gray, or absent. Soluble pigment yellow to almost none.

Gelatin: Cream-colored to yellow to orange-yellow ring on surface. Aerial mycelium cream-colored, straw-green to gray, or absent. Soluble pigment brownish to yellow, or absent. Liquefaction varies from slow to rapid.

Potato: Growth abundant, lichenoid, cream-colored to brownish to orange. Aerial mycelium white, cream-colored, gray to yellow. Usually no soluble pigment; occasional yellowish-orange pigment.

Milk: Surface growth abundant or thin gray to black ring. Aerial mycelium white to gray or absent. Milk not coagulated but peptonized, the rapidity depending on extent of growth.

Antagonistic properties: Some members of the group produce highly important antibiotics, such as the tetracyclines, that have found extensive application in chemotherapy and in food preservation (Kochi *et al.*,1952).

A careful study of the literature reveals the fact that a large number of species found in nature belong to this series. Some of them have been well recognized and described. Various others may be added, but many have been only insufficiently described. On the basis of the recognized information, the *S. flavus* series may be said to include the

following species: *S. flavus, S. flavovirens, S. flavogriseus, S. chrysomallus, S. celluloflavus,* and *S. viridans.*

IV. Series *Ruber*

Characteristic Properties

a. Sporophores straight or spiral-shaped.

b. Substrate growth pink, red to red-orange to purple-red; pigment insoluble. Aerial mycelium thin, rose-white.

c. Melanin-negative.

The *Ruber* series comprises a large, highly heterogeneous group of organisms. Members of this series have been known since 1888, when Macé described an organism under the name of *Cladothrix rubra.* Numerous other cultures under different names were later placed in this group.

The *Ruber* series is characterized by a bright red, red-orange, or rose-red substrate growth, the color depending on the composition of the medium and on conditions of cultivation. The cultures may show considerable variation in color of the substrate mycelium, from purple-red to light rose. The pigment is usually not excreted into the medium, unless the latter contains fatty substances in which the pigment is soluble. The aerial mycelium is not well developed; it is usually produced on synthetic media as a thin, rose-white cover, or it is formed only in isolated sectors or spots. The sporophores are straight or spiral-shaped; the spores are spherical to oval, 0.7 to 0.8 by 0.8 to 1.0 μ.

The members of the *Ruber* series are not very strongly proteolytic or diastatic. Sucrose is readily inverted. Some of the species belonging to this series are active producers of antibiotics.

Baldacci did not list a *Ruber* series, but one designated as "*roseus,*" which is close enough to be considered similar to it. Another was designated as "*melanosporeus,*" which is also close to the "*ruber*" series. Gause *et al.* (1957) divided the series into three subgroups on the basis of structure of the sporophores, namely, spiral-shaped, straight, and tuft-forming; the last apparently includes verticil-forming types.

Some of these organisms may be considered as forms intermediate in transition to the true chromogenic types. Among the forms closely related to this series, one may include, for example, *S. melanocyclus, S. melanosporeus, S. melanogenes,* and possibly also *S. erythrochromogenes, S. roseochromogenes,* and *S. purpureochromogenes.* The *Ruber* series is also related to the *Fradiae* and *Flavus* series, notably through such species as *S. roseoflavus* and possibly *S. microflavus.*

Certain forms that may be considered as species of *Nocardia* are frequently included in this and in the next series. Sometimes even a new series is created for them, as was done by Baldacci (1942) for "*madurae.*"

S. albosporeus may be considered as a subgroup of the *Ruber* series. It is characterized by the formation of a rose-colored or red to brown substrate growth and a white aerial mycelium. Cultures belonging to this subgroup are characterized by strong proteolytic activity and by weak diastatic action. The sporophores are straight, with some close spirals. The first representatives of this subgroup were isolated by Krainsky in 1914 and by Waksman and Curtis in 1916.

The separation of members of this series on the basis of carbon utilization has been suggested by Zähner and Ettlinger (1957), as shown in Table 15.

Although a large number of species found in the literature may be included in the *Ruber* series, only a few have been sufficiently described. It is sufficient to mention *S. ruber, S. niveoruber, S. albosporeus,* and *S. erythraeus.*

Table 15

Utilization of carbon sources by a group of closely related Streptomyces *species* (Corbaz *et al.*, 1957)

Culture	L-Xylose	L-Rhamnose	D-Fructose	Saccharose	Lactose	Raffinose	Inulin	D-Mannitol	D-Sorbitol	Mesoinositol	Salicin
S. purpurascens	+	+	+	+	+	+	(+)	+	(−)	+	+
S. bobiliae	+	+	+	+	+	+	+	(−)	(−)	(+)	+
S. cinereoruber	+	−	(−)	−	+	−	−	−	(−)	−	+
S. cinereoruber var. *fructofermentans*	+	(+)	+	(+)	+	−	(−)	+	+	+	+

+ = good growth; (+) = weak growth, questionable carbon utilization; (−) = very weak growth; − = no growth.

V. Series *Viridis*

Characteristic Properties

a. Sporophores straight or spiral-shaped.

b. Growth at first colorless, becoming green to dark green. Aerial mycelium white to gray to light green to light blue.

c. Melanin-negative.

d. Soluble pigment absent or greenish.

The species included in this series show considerable overlapping with the species included in the chromogenic series, such as *S. viridochromogenes* (syn. *A. viridis* (Lombardo-Pellegrino) Baldacci).

Various other organisms that might be included in this series have been described. It is sufficient to list *S. alboviridis*, *S. griseoviridis*, and *S. dassonvillei*.

The following organisms may be included in the *Viridis* series: *S. viridis*, *S. prasinus*, *S. hirsutus* and *S. prasinopilous*.

Several forms described by Gause *et al.* (1957) could be included in this series, notably *A. malachiticus* and *A. olivaceoviridis*.

VI. Series *Violaceoruber*

Characteristic Properties

a. Sporophores produce spirals. Spores spherical to oval. Surface of spores smooth.

b. Substrate growth colorless, becoming red, later blue. Aerial mycelium white to gray with bluish tinge.

c. Melanin-negative. Soluble red pigment in acid media, changing to blue in alkaline.

A number of organisms belonging to the genus *Streptomyces* are able to produce a blue pigment when grown on certain media (Tables 16, 17). This pigment is either retained in the substrate mycelium or is readily dissolved in the medium; it is frequently accompanied by a dark chromogenic pigment. The color of the pigment ranges, therefore, from light blue to dark blue or violet, and to almost black. The soluble pigment frequently changes in color with a change in reaction of medium, from red at an acid reaction to blue at an alkaline reaction. Because of this change in the color of the pigment, various names, indicating the red and blue color combinations, have been used to describe the species, such as "*violaceus*," "*violaceoruber*," "*violaceoniger*," "*tricolor*," and "*pluricolor*." The species capable of producing blue pigments are divided here into two distinct subgroups: *S. violaceoruber* and *S. violaceoniger*. The first comprises the forms that produce a litmus-like pigment, changing from red in acid media to blue in alkaline; the second includes those forms that produce violet to dark blue to almost violet-black pigments on synthetic and organic media.

TABLE 16

Streptomyces *species, producing a blue pigment* (Kutzner and Waksman, 1959)

Organism	Color of aerial mycelium	Spirals	Spore surface	Melanin pigment	Author
S. caeruleus	Light grayish-blue	−		−	Baldacci, 1944
S. coelicolor	Grayish-yellow	−	Smooth	−	Müller, 1908
S. cyaneofuscatus	Greenish-grayish-yellow	−		+	Gause *et al.*, 1957
S. cyaneus	Bluish-gray to blue	+	Spiny	+	Krassilnikov, 1949
S. cyanoflavus	Greenish-brownish-gray	−		+	Funaki *et al.*, 1958
S. litmocidini	Gray, sometimes with brownish tinge	−; seldom +			Gause *et al.*, 1957
S. novaecaesareae (= *A. violaceus caesari*)	White with purple tinge due to substrate mycelium	+		−	Waksman and Curtis, 1916; Waksman, 1919
S. pluricolor	Whitish-gray	+			Berestnew, 1897; Krassilnikov, 1949
S. tricolor	Light brown to light gray	+			Wollenweber, 1920
S. violaceoruber	Ash-gray	+	Smooth	−	Waksman and Curtis, 1916; Waksman, 1919
S. olivaceus	Ash-gray	−	Smooth	−	Corbaz *et al.*, 1957
Streptomyces sp. No. 169	Mouse-gray			+	Kurosawa, 1951

The first organism belonging to this series was isolated, in 1891, by Rossi-Doria and described as *Streptothrix violacea*. It was later studied by Gasperini (1894), and by Sanfelice (1904) as one of the three important constituent groups of actinomycetes.

Baldacci (1942) designated the series as "*violaceus*," which he did not differentiate, however, from the subgroup designated here as *violaceoniger*. Gause *et al.* (1957) created a new series, "*roseoviolaceus*," which logically belongs in this series; they also included in their series "*violaceus*" a variety of other forms that logically belong to this series. Ettlinger *et al.* (1958) designated as *azureus* the light blue pigmented forms.

This series is not known for the production of any important antibiotics, although coelicolorin has been reported for cultures of *S. violaceoruber* and chartreusin for *S. chartreusis*.

The following species may be included in this series: *S. violaceoruber*, *S. novaecaesareae*, *S. cyanofuscatus*, and *S. litmocidini*.

Some of the melanin-producing forms, such as *S. violaceochromogenes*, may also be included in this series. Many of the forms described by Gause *et al.* (1957) also belong here. These include *A. coerulescens*, *A. glaucescens*, *A. coeruleorubidus*, *A. bicolor*, *A. coeruleofuscus*, *A. violaceorectus*, *A. pranicolor*, *A. litmocidini*, *A. viridoviolaceus*, *A. griseorubiginosus*, *A. griseoruber*, *A. cinnabarinus*, and others.

VII. Series *Fradiae*

Characteristic Properties

a. Sporophores usually straight; occasional loops and spirals.

b. Substrate growth yellow-orange to orange. Aerial mycelium seashell-pink, especially on potato agar and on glucose-asparagine agar.

c. Melanin-negative.

This represents a fairly large group of organisms, widely distributed in nature. *S. fradiae* was first isolated and described by

TABLE 17

Blue-pigmented substances produced by actinomycetes (Kutzner and Waksman, 1959)

Preparation	Organism	Melting point	Solubility	Author
		°C		
Amylocyanin	*S. coelicolor*		In water and in dimethylformamide; insoluble in other solvents	Müller, 1908
Litmocidin	*Nocardia cyanea*	144–146	Slightly soluble in water at an acid reaction and extracted from it by ethanol, ether, or amylacetate	Gause, 1946; Brazhnikova, 1946
Coelicolorin	*S. coelicolor*	142–146	Very soluble in acetone, ethylacetate, or chloroform; soluble in ethanol, methanol, benzene, or ether; insoluble in petroleum ether	Kominami, 1949; Hatsuta, 1949
Cyanomycin	*S. cyanoflavus*	128	Extracted from water at an alkaline reaction by chloroform or methylenechloride	Funaki *et al.*, 1958
Granatacin	*S. olivaceus*	204–206	Extracted from water at an acid reaction by acetone; soluble in ethylacetate, and dimethylsulfoxide; insoluble in petroleum ether	Corbaz *et al.*, 1957
Actinorhodin	*S. coelicolor*	270 (decomp)	Soluble in pyridine, piperidine, or phenol; weakly soluble in dioxane or acetone; insoluble in ether, CS_2, CCl_4, or petroleum ether	Brockmann *et al.*, 1947, 1950, 1955
Streptocyanin	*Streptomyces* sp.	290–300 (decomp)	Soluble in acetone, dioxane, or pyridine	Tonolo *et al.*, 1954
Anthocyanin	*S. violaceoruber*		Extracted with hot or cold water and dilute alcohol	Kriss, 1936
Anthocyanin	*S. coelicolor*		Extracted with hot or cold water and dilute alcohol	Kriss, 1937
Anthocyanin	*S. violaceoruber*			Frampton and Taylor, 1938
Hydroactinochrome	*Streptomyces* sp., producing violet growth and pigment		Soluble in water	Kriss, 1936
Lipoactinochrome			Insoluble in water	Kriss, 1936

Waksman and Curtis (1916). Of the two neomycin-producing cultures isolated by Waksman and Lechevalier in 1949, one formed no spirals and thus agreed with the original description of the organism; the second produced some spirals of the closed type. Differences were also observed in the shade of color of the aerial mycelium on synthetic media.

Some of the strains of *S. fradiae* were found capable of producing certain antibiotics, notably members of the neomycin complex, as well as the antifungal agent fradicin. Several other species reported in the litera-

ture appear to be related to the *Fradiae* series. Baldacci *et al.* (1953) at first did not recognize this organism as representing a distinct series, and apparently considered it as a member within the "*roseus*" series. Later, however, Baldacci and Comaschi (1956) gave it series characteristics.

Gause *et al.* (1957) divided the *Fradiae* series, on the basis of spiral formation, into two subgroups: one, spiral-forming, comprising *A. roseoflavus*; the other, nonspiral-forming, comprising *A. fradiae* proper; other species and varieties were included in both subgroups. Most of the members of a new series, designated as "*fuscus*," could also be considered as members of the *Fradiae* series.

Waksman and Scotti (1958) divided the *Fradiae* series into three subgroups. These were described briefly as follows:

I. Substrate growth on synthetic media thin, smooth, colorless, almost entirely limited to the surface of the medium; occasionally colored orange-yellow. Aerial mycelium light pink, seashell-pink, or salmon-colored. Some strains produced little if any growth on synthetic media. Best sporulation took place on potato agar and on glucose-asparagine agar. On organic media, growth was smooth to wrinkled, yellowish or orange-yellow to orange-brown; aerial mycelium, if present, was white to seashell-pink. On certain media, a soluble, pink to salmon-colored pigment was produced. Morphologically, all strains formed a straight aerial mycelium; some cultures, however, were able to form hooks and loops, and even occasional spirals, on certain media. These strains were considered as representing typical *S. fradiae* proper.

IIa. On synthetic media, substrate growth thin, colorless, limited almost entirely to the surface of the medium; aerial mycelium white. On organic media, growth cream-colored to yellowish; aerial mycelium thin, white to grayish-white. On yeast-glucose agar, growth orange to brownish to greenish; aerial mycelium white. Abundant spirals were found in the aerial mycelium.

IIb. On synthetic media, growth very poor. On organic media, growth generally poor; growth best on yeast-glucose agar. No aerial mycelium was formed.

A detailed characterization of subgroup I is given in Table 18. Among the other species apparently closely related to this section is *S. kanamyceticus* (Okami and Umezawa).

The following species may be included in the *Fradiae* series: *S. fradiae*, *S. luridus*, *S. albosporeus*, perhaps also *S. roseus* and *S. fuscus*.

VIII. Series *Griseus*

Characteristic Properties

a. Sporophores straight, produced in tufts. Spores oval; surface smooth.

b. Growth colorless to olive-buff. Aerial mycelium water-green to grass-green to gray.

c. Melanin-negative.

d. Strong proteolytic activities. Produce a variety of antibiotics.

An organism, under the name of *A. griseus*, was first isolated and described by Krainsky in 1914. Its substrate growth on artificial media was colorless; only a small amount of yellowish soluble pigment was produced. The aerial mycelium was of a green-gray color on both organic and synthetic media. When the concentration of nitrogen in the medium was increased to 0.005 per cent, the aerial mycelium became white. The culture was only weakly proteolytic.

Soon afterward, in 1915, Waksman and Curtis isolated several cultures of what appeared to be the same organism, the comparison with Krainsky's description being based primarily on the color of the aerial mycelium. Since this work was done during the years of World War I, Krainsky's original strain could not be obtained for comparative studies. The new culture was des-

TABLE 18

Morphological and cultural characteristics of Streptomyces fradiae (Waksman and Scotti, 1958)

Characteristics	Culture No.						
	3535	3554	3556a	3556b	3572	3594	3719
Structure of aerial mycelium	Hyphae straight; occasional hooks and loops on some media; spores rod- to oval-shaped	Hyphae straight; loops and short spirals on some media; spores rod- to oval-shaped	Hyphae straight; hooks on old cultures; spores rod- to oval-shaped	Hyphae with some loops and short spirals; spores rod- to oval-shaped	Hyphae straight; rare hooks; spores rod- to oval-shaped	Hyphae straight; spores rod- to oval-shaped	Hyphae straight or wavy; short spirals abundant on some media. Spores rod- to oval-shaped
Glucose-asparagine agar	Growth yellow-orange; aerial mycelium white or pink	Growth yellow-orange; aerial mycelium pink in old cultures	Growth orange-yellowish; aerial mycelium powdery	Growth yellowish; aerial mycelium powdery, seashell-pink	Growth orange to yellowish; aerial mycelium scant, pale pink	Growth smooth, orange-yellow; pink sporulation in old cultures	Growth smooth, yellow-orange; aerial mycelium white or pink on edge.
Synthetic agar	Growth very poor; aerial mycelium pink	Growth very poor; aerial mycelium pink	Growth very poor; aerial mycelium pale pink	Growth poor; aerial mycelium pink	No growth	Growth very poor; aerial mycelium pink	Growth very poor; aerial mycelium pink
Nutrient agar	Growth smooth and yellowish; aerial mycelium chalk-white with pink shade	Growth colorless to yellowish; aerial mycelium scarce, white	Growth smooth, yellowish; aerial mycelium white	Growth smooth; aerial mycelium white with pale pink shades	Growth smooth, opaque, colorless; aerial mycelium scant	Growth smooth, colorless to deep yellow; aerial mycelium poor, white	Growth smooth, yellowish; aerial mycelium white, with pinkish shade at edge
Potato agar	Growth smooth, flesh-pinkish in reverse; aerial mycelium pink	Growth smooth; aerial mycelium pink	Growth smooth; aerial mycelium pink	Growth smooth; aerial mycelium pink, with some white spots	Growth colorless; aerial mycelium white with pink sporulation on edge	Growth smooth, colorless; pink sporulation on edge	Growth smooth, yellow; aerial mycelium white, later pink
Potato plug	Growth light brown; aerial mycelium scarce, chalk-white	Growth light brown or light orange, later brown; aerial mycelium white	Growth light brown or brownish orange; aerial mycelium chalk-white, with pinkish shades	Growth light orange-brown; aerial mycelium ivory white, later chalk-white with pinkish shades	Growth rusty brown; no aerial mycelium	Growth rusty brown-orange; no aerial mycelium	
Yeast glucose agar	Growth orange-salmon, later brownish yellow; aerial mycelium scarce, white or pinkish; soluble pigment pale orange	Growth orange-salmon, later orange-brown; aerial mycelium white. Trace of pink-orange soluble pigment	Growth salmon-colored; aerial mycelium white or pink on edge	Growth salmon-pink, with orange reverse; aerial mycelium white or pink; some pinkish soluble pigment	Growth opaque, orange; aerial mycelium white with pink shades; soluble pigment salmon-pink	Growth salmon-pink to yellowish, later brownish; soluble pigment of same color	Growth good, yellow-orange; aerial mycelium scarce, white
Gelatin	Liquefaction complete; some aerial mycelium and yellow pigment	Liquefaction complete; light yellow pigment late	Liquefaction; light yellow pigment late	Liquefaction complete; light yellow pigment late	Liquefaction complete; light yellow pigment late	Liquefaction; brownish-yellow pigment	
Antibiotic activity	Against gram + and gram − organisms and *Candida*; none against *Pseudomonas*	Against gram +, gram −, and *Candida*; some against *Pseudomonas*	Against gram +, gram −, and *Candida*; none against *Pseudomonas*	Against gram +, gram −, and *Candida*; none against *Pseudomonas*	Against gram +, gram −; none against *Pseudomonas*	Against gram +, gram −, and *Candida*; scarce against *Pseudomonas*	Against gram + and gram − bacteria

ignated as *A. griseus* Krainsky, although certain marked differences were observed between the two isolates.

Since no type culture of Krainsky's organism was available for comparison to any investigator, all the subsequent descriptions were based upon the Waksman and Curtis culture, which was distributed to all collections in the world.

In 1919, Waksman amended the description of Krainsky, as follows: "This organism was isolated numerous times from the soil. The name *A. griseus* was used before by Krainsky so that the description of the latter is itself an amendment. Although this organism was originally identified with the organism described by Krainsky under the same name (from description only, without any actual comparison of cultures), this identification should be, therefore, corrected. The culture described here possesses a very strong proteolytic power, while Krainsky stated that his culture was not strong proteolytically."

The differences between the two cultures can be briefly summarized as follows: *A. griseus* Krainsky produced a greenish-gray to dark gray aerial mycelium, with a greenish-yellow soluble pigment in older cultures; growth on potato was grayish, with white-gray aerial mycelium; Krainsky never studied the morphology of his organism, except for the shape (oval) and size of the spores. *A. griseus* Waksman and Curtis produced a water-green to yellowish-green aerial mycelium; the sporophores were straight and were formed in tuft-like masses; growth on potato was yellowish, wrinkled, and without any soluble pigment.

In spite of these differences, Waksman hesitated at first to change the name of the culture which he and Curtis first isolated. This hesitation was due partly to the fact that the organism was found to undergo considerable variation upon continued cultivation on artificial media. The substrate, the temperature of incubation, the length of the incubation period, the amount and nature of inoculum, all tended to exert an influence upon the morphological and cultural characteristics of the organism. At one time milk was clotted at 37°C in 2 days and then peptonized; at another time, under the same conditions, clotting of the milk required 5 to 6 days; at still another time, the milk in some tubes was not clotted at all but was rapidly peptonized. There were other recognizable changes or variations. Drechsler, studying the morphology of the Waksman and Curtis culture, found that the aerial mycelium showed proliferation of fertile branches at moderately close intervals along the axial hyphae, thus suggesting tuft formation. This phenomenon alone would have definitely indicated that the culture should have been identified as a distinct species.

In August 1943, in the laboratories of the Department of Microbiology of the New Jersey Agricultural Experiment Station, a culture was isolated which produced the highly important antibiotic designated as streptomycin. Upon careful examination, this culture was found to be similar to the *Actinomyces griseus* described by Waksman and Curtis in 1916. Since, in the meantime, Waksman and Henrici had proposed that the generic name for the sporulating forms of actinomycetes be changed from *Actinomyces* to *Streptomyces*, the organism was named *Streptomyces griseus*. This name has been universally recognized, since 1944, as the official one for the streptomycin-producing organism, and has been so designated in numerous other collections throughout the world. A detailed description of this species was published in 1948 (Waksman, Reilly and Harris).

Baldacci *et al.* (1954) subjected the *Griseus* series to a detailed study. They recognized that this representative species had come to the fore as a result of the important role that it played in the production of antibi-

otics. They emphasized that although first listed by Krainsky in 1914, *S. griseus* was amended and described in detail by Waksman in 1919. They further added:

"If we examine the characteristics given by Krainsky we are led to link this species with *A. viridis*. This conclusion appears still more logical when we study the coloured tables prepared by Krainsky. However, in view of the impossibility of comparing Krainsky's original strain and the difficulties that would arise if one did not accept Waksman's amendment for a species so generally studied in laboratories, it appears advisable to take as definite the characteristics specified by the American author and given in Bergey's Manual .... Numerous strains have been isolated by us and compared, with satisfactory results, with Waksman's strains. . . .There is a considerable body of literature dealing with this species which has a faculty for mutation."

Baldacci and Comaschi later (1956) wrote:

"The examination of Krainsky's description and colored pictures would suggest that this species belongs to *A. viridis* Lombardo Pellegrino (1903). The comparison between Krainsky's and Waksman's descriptions gives evidence—as even Waksman has partially pointed out—to the difference of proteolytic activity and, according to our opinion, the very important difference of the color of the sporulating colonies which are greenish in Krainsky's description. If we accept Waksman's correction of the species and compare his descriptions with our strains, we find a perfect identity. Since it is impossible to compare the original strain of Krainsky with the others, the acceptance of the correction proposed by Waksman offers the advantage of maintaining the name "*griseus*" for an actinomycete so largely spread out and studied in laboratories, so that we agree with it according to this meaning."

Many other cultures of *S. griseus* have since been isolated from soils, river muds, animal excreta, water, dust, and other natural substrates. Not all of them were found capable, however, of producing streptomycin; the majority of these cultures were either inactive or produced other antibiotics, such as cycloheximide, grisein, streptocin, actinomycin, and candicidin. Some of the cultures yielded a mixture of streptomycin with other antibiotics. The ability to form streptomycin was at first considered as a strain, rather than a species, characteristic; later, however, it was decided (Waksman, 1959) to raise *S. griseus* to the status of a series and the streptomycin-producing strains to a species status, *Streptomyces griseus*, Waksman and Henrici.

Several procedures were developed for the isolation from natural substrates and for the identification of streptomycin-producing strains of *S. griseus*. These methods were based on certain physiological properties of the organisms and on the nature and activities of the streptomycin formed by them:

1. Tolerance to fairly high concentrations of streptomycin in the medium. When a soil or other natural material was plated out on a medium containing 50 mg of streptomycin per liter, the great majority of bacteria and actinomycetes failed to develop on the plate. Most of the actinomycete colonies were found to be of the *S. griseus* type.

2. Ability of certain resistant strains of test bacteria to grow in the presence of streptomycin.

3. Sensitivity to a specific actinophage. When cultures of *S. griseus* are tested for their sensitivity to a specific actinophage which is active only upon the streptomycin-producing species, the inactive forms or those producing other antibiotics can be easily eliminated.

4. Utilization of streptomycin-dependent strains of bacteria in testing for streptomycin. When a culture of *S. griseus* or of another organism suspected of producing

streptomycin was finally selected and grown in a liquid medium, the streptomycin-like nature of the antibiotic could be established by adding the culture filtrate to a nutrient broth and inoculating the latter with a streptomycin-dependent strain of *Escherichia coli* or of some other bacterium. Growth of the bacterium definitely established the fact that the unknown antibiotic was streptomycin.

5. Cross-streaking the unknown cultures on a suitable agar medium toward known streptomycin-producing cultures. The latter exerted only a slight inhibiting effect upon the unknown streptomycin-producers.

Usually some soil or other material is plated on ordinary agar media favorable to the development of actinomycetes; colonies were picked and tested. The *S. griseus* colonies could easily be recognized by the pale green to grayish-green shade of their aerial mycelium. A suitable agar medium can also be seeded with living cells of a nonpathogenic strain of *Mycobacterium tuberculosis* and various dilutions of soil used for plating purposes. The plates are first incubated at 28–30°C for 2 or 3 days, to enable the actinomycetes to develop. This is followed by further incubation of the plates at 37°C for the development of the test bacterium. Colonies that have the capacity of inhibiting growth of the bacterium are found to be surrounded by clear zones.

The antibiotic potency of an active culture of *S. griseus* was found to be fairly constant, in spite of the ability of the culture to give rise to inactive variants. Highly active strains tend to retain their relatively superior streptomycin-producing potency, whereas poor strains usually remain weak producers of this antibiotic. For the commercial production of streptomycin, however, it is essential to select continuously the most active strains.

Since the streptomycin-producing culture isolated in 1943 was found to be identical with the one described by Waksman and Curtis, it must be considered in the light of that description. The same is true of the *S. griseus* strains isolated later and found to be able to produce grisein, candicidin, viomycin, and actinomycin. There are certain differences in the cultural and biochemical properties of the various strains belonging to the *Griseus* series, especially in their ability to produce various antibiotics and in their sensitivity to different phages. This justifies the separation of the group into several distinct species.

The morphological and cultural properties of certain cultures belonging to the *Griseus* series are given in Table 19.

Characterization

The *Griseus* series is characterized by certain morphological and cultural properties that make possible its identification and ready distinction from other groups belonging to the genus *Streptomyces*. As more and more cultures of *S. griseus* were isolated, it became recognized that this is a large series of organisms, the members of which vary greatly in their physiological properties and in their ability to produce various antibiotics.

Waksman and Curtis described *S. griseus* as producing on sucrose nitrate agar a thin, spreading growth, developing deep into the medium, at first colorless, then turning olive-buff. This color may be lost on successive transfers. The aerial mycelium is thick, powdery, water-green in color. No soluble pigment was observed; the reverse of the growth became brownish in 24 days. On gelatin, at 18°C, *S. griseus* produced a greenish-yellow or cream-colored growth developing deep into the substrate; the aerial mycelium was white-gray with a greenish tinge. There was no soluble pigment; liquefaction of the gelatin was rapid. The culture was capable of utilizing a variety of carbohydrates, including pentoses, hexoses, sugar alcohols, and organic acids. It was also able to obtain its

TABLE 19

Morphological and physiological properties of certain strains and one mutant of Streptomyces griseus

Strain of organism	Morphology	Synthetic agar		Glycerol agar		Gelatin*	Potato	Antibiotic properties
		Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	
1915 W and C (N. J. strain)	Sporophores long, formed in tufts, no spirals	Cream-colored	Powdery, water-green color	Cream-colored	White to greenish-yellow	Cream-colored or greenish-yellow	White to greenish-yellow	0†
1915 W and C (Holland strain)	Sporophores long, formed in tufts, no spirals	Cream-colored, turning olive-buff	Powdery, water-green color	Cream-colored	White to greenish-yellow	Greenish-yellow or cream-colored	Grayish	++
1943 Streptomycin producer	Tufts, no spirals	Cream-colored, turning olive-buff	Powdery, water-green color	Cream to olive-green	Cream-colored	Greenish yellow or cream-colored	Greenish tinge	++++
Grisein producer	Tufts, no spirals	Cream-colored	Light gray to greenish	Cream-colored	Cream-colored to greenish	Greenish-yellow	Grayish	++
Rhodomycetin producer	Tufts, no spirals	Vinaceous	White to gray	Carmine-red	Gray	+++	Gray	++

* Brownish pigment produced by some strains.

† Mutant of this culture produced an antibiotic.

nitrogen from a variety of compounds, including both inorganic and organic forms.

In studies of streptomycin-producing strains, Carvajal (1946) characterized the morphology and life cycle of *S. griseus* in greater detail. The substrate mycelium when young is well branched, typically in a monopodial form. Transverse septa are formed in virtually all cases in the delimitation of the reproductive cells. Reproduction occurs by means of unicellular asexual spores and conidia, which are exogenously borne in chains on the aerial mycelium. The spores are of various shapes: barrel, oval, bean, spherical, and cylindrical. Differences in shape and size are found often, even among the spores of the same chain. Mature aerial spores often show small fragments of transparent film adhering to the outside wall. The spores germinate at one end or at both ends, usually from the points at which they are attached to the adjacent spores or to the hypha. Hyphal fusions and germ tube fusions also can be observed. Carvajal reported that he had demonstrated a nucleus in the germ tubes of *S. griseus* in the young mycelium and in the developing spores. The nuclei were said to be well distributed throughout the cytoplasm of the mycelium; the spores may be uninucleate or multinucleate.

Gottlieb and Anderson (1947) studied the course of spore germination and of development of the mycelium in submerged cultures of *S. griseus*. The exact time of spore germination was difficult to determine, only an elongation of the spores being observed. After 6 hours, the mycelium was found to consist of some small hyphae and of longer branched hyphae which tended to develop into masses of mycelium consisting of a dense solid center and a periphery of branched radiating hyphae. Within 24 to 30 hours, the entire body of the medium was filled with these mycelial clumps. The culture appeared viscous at this stage. After 48 hours, the mycelium began to fragment, and spores were produced. At 84 hours, definite lysis of the mycelium took place; the dense central core of the masses of growth disintegrated into granular pieces.

Measurement of viscosity and weight of mycelium revealed an increase which reached

a maximum at 24 to 30 hours, followed by a decrease up to about 96 hours; a gradual leveling of growth then took place.

Growth of *S. griseus* in stationary cultures reaches a maximum in 10 days, whereas maximum growth in submerged cultures is usually attained in 3 to 5 days. This is followed by lysis of the mycelium. Growth of the organism is accompanied by a gradual rise in pH value of the culture and in the ammonia and amino nitrogen content; the total nitrogen in the mycelium tends to be higher during the active stages of growth. The production and accumulation of streptomycin parallel the growth of the organism, reaching a maximum when lysis just sets in; this is followed by a decrease when the rate of lysis reaches a maximum.

Metabolism

The metabolic changes of *S. griseus* in a glucose-peptone-meat extract medium have been found by Dulaney and Perlman (1947) to fall into two phases. During the first phase, the organism grows rapidly and forms extensive mycelium; this is accompanied by a reduction in the quantity of soluble constituents in the medium, namely, the nitrogen, the inorganic phosphate, and the available carbohydrate; the quantity of lactic acid present is first increased and then utilized to some extent; the oxygen demand is high, and the Q_{O_2} values may reach 150; little streptomycin is produced; the soluble carbon content of the medium during the growth phase rapidly falls as the glucose is utilized; about 50 per cent of the carbon appears to be unavailable to the organism during the first stage; the nitrogen content of the mycelium varies with age. During the second or autolytic phase of growth considerable lysis sets in; streptomycin is produced actively, and the pH of the medium rises; the quantity of mycelium is decreased as a result of lysis; the lactic acid content remains more or less constant, as does the soluble carbon content of the medium; the oxygen demand slowly decreases; the ammonia nitrogen, soluble nitrogen, and inorganic phosphate contents of the medium rise rather markedly, paralleling the autolysis of the cells.

Ammonium compounds, but not nitrates are favorable sources of nitrogen for growth and streptomycin production. *S. griseus* rapidly assimilates phosphate in a phosphorus-poor medium. An excess of phosphorus has a depressive effect both upon growth of the organism and upon streptomycin production.

The supplementary addition of amino acids or of more complex organic compounds has been found to stimulate production of streptomycin. Eiser and McFarlane (1948) found that, of the amino acids, histidine is essential for both mycelial growth and streptomycin production; inositol also increased the yield of both; valine favored the latter, and aspartic or glutamic acid the former. If the salt concentration is low, most of the streptomycin will be found in the mycelium, thus suggesting that streptomycin is a product of intracellular synthesis. Woodruff and Ruger (1948) reported that yields as high as 1 g of streptomycin per liter are produced by *S. griseus* in media containing proline as the only source of nitrogen.

The ability of *S. griseus* to form an enzyme (mannosidostreptomycinase) which decomposes mannosidostreptomycin into streptomycin and mannose has been recently demonstrated. This enzyme is not produced by other actinomycetes or fungi (Volume I, p. 187).

On a dry basis, the mycelium of *S. griseus* contains about 16 per cent ether-soluble material and about 37 per cent cold water-soluble substances. Little study has been made of the specific chemical composition of these and other fractions.

Stokes and Gunness (1946) grew *S. griseus* in stationary cultures in a nutrient medium

containing 0.5 per cent meat extract and 1 per cent glucose. The cell material was dried and then hydrolyzed by acid or alkali. The amino acid composition of this material, on a percentage basis of the dry material, was as follows: total nitrogen, 9.14; histidine, 0.84; arginine, 2.90; lysine, 2.13; leucine, 3.73; isoleucine, 1.49; valine, 3.40; methionine, 0.55; threonine, 2.33; phenylalanine, 1.67; tryptophan, 0.62.

In addition to the two forms of streptomycin, *S. griseus* produces several other antibiotics. Ether extracts from the mycelium of the organism yield a substance designated as streptocin, which is active against gram-positive bacteria but not against gram-negative forms. Another antibiotic, designated as cycloheximide, can be isolated by extracting the crude submerged culture with chloroform, evaporating the extract, and dissolving the residue in methanol. Cycloheximide is not active against bacteria but has strong antifungal properties; it is particularly active against yeasts.

Varieties and Mutants

The *Griseus* series represents a large, widely distributed, and variable group of organisms. It has long been recognized (Waksman, 1959) that this series should be divided into several species. The formation of different antibiotics by the various species offers an excellent supplementary basis for such subdivision. The many cultures isolated and studied in detail can thus be classified into five distinct species.

1. *Streptomyces griseus* Waksman and Henrici. This comprises strains of *S. griseus* which produce streptomycin; they also produce cycloheximide.

2. *Streptomyces griseinus* Waksman. Strains of *S. griseus* which produce grisein or grisein-like substances. These strains are as a rule resistant to actinophage. Benedict and Lindenfelser (1951) demonstrated that a majority of streptomycin-producing strains of *S. griseus* form a green soluble pigment in calcium malate medium and a yellow pigment in calcium succinate medium; on the other hand, the grisein-producing strains of this organism do not form any green or yellow pigments in these media, although they show the typical greenish pigmentation of the aerial mycelium.

3. *Streptomyces coelicolor* (Müller) *emend.* Kutzner and Waksman. This species comprises strains which produce the antifungal agent candicidin but no antibacterial substance. The first organsim belonging to this species was isolated by Müller (1908), and designated *Streptothrix coelicolor*. It produced a blue pigment similar to that formed by a diphtheroid organism which he called *Bacillus coelicolor*. The culture was a typical *Streptomyces* and formed concentric rings in its aerial mycelium. It developed well at room temperature and at 36°C. It grew on gelatin, with gradual liquefaction but without pigmentation. On agar media containing 5 to 10 per cent dextrin, but not in glycerol media, a brown pigment was formed. The culture formed no aerial mycelium on ordinary agar media, unless serum, glycogen, dextrin, or starch was added. When glucose, sucrose, arabinose, or other sugars were used, no aerial mycelium was formed.

Müller emphasized two important activities of *S. coelicolor:* It possessed antagonistic properties, and it was active against *Oidium lactis*. Müller was thus one of the first to demonstrate activities that were to make the whole group of actinomycetes famous. Müller also studied the pigment extensively; he called it amylocyanin; it is produced best on potato media when grown at 30°C, but not at 36°C.

4. *Streptomyces californicus* Waksman and Curtis. This species comprises strains which produce viomycin, active against gram-positive bacteria only. Burkholder *et al.* (1955) classified these organisms as strains of *S. griseus* var. *purpureus*. Waksman (1958)

TABLE 20

Comparison of cultural characteristics of four strains of Streptomyces californicus *with* Streptomyces griseus *on five different media* (Burkholder *et al*, 1955)

Agar medium	Mycelium	*S. floridae*	*S. californicus*	*S. puniceus*	*S. vinaceus*	*S. griseus* 3475
Glycerol asparagine	Substrate	White to light gray to slight purple	White to light gray to purple	Purple	White to light gray to slight purple	White to light yellow
	Aerial	White	White	White to light gray-green	White to light gray-green	White to light gray-green
Glucose tryptone	Substrate	Black with slight purple tinge	Gray to slight black	Gray to black	Gray to black	Light gray-yellow
	Aerial	White to slight gray-pink	Slight growth, white	Light gray-pink to light gray-green	Light gray-pink to slight light gray-green	White to gray-pink
Starch synthetic	Substrate	Purple	Light gray to slight purple	Purple	White to light gray to slight purple	Light yellow-gray
	Aerial	White to light gray-green	White	White to light gray-green	Light gray-green	Light gray-green
Calcium malate	Substrate	White to light yellow	White to light yellow	White	White	Gray-yellow to slight light brown
	Aerial	White	White	Light gray-green	White	White to slight gray-green
Nutrient	Substrate	White to slight light yellow	White to slight light yellow	White to light yellow	White to light gray-yellow	Light gray-yellow
	Aerial	White	White	White	White to slight light gray-green	White to slight gray-green

suggested that they be raised to the status of a species within the *S. griseus* section. A further study of this species points to its identity to *S. californicus*, which has priority in species designation.

5. *Streptomyces chrysomallus* (Lindenbein) Waksman. These comprise strains which produce actinomycin. Welsch *et al.* (1957) studied 51 cultures of *Streptomyces* for their susceptibility to seven actinophages; 43 of the strains produced actinomycin and eight represented nonactinomycin-producing strains of *S. griseus*. Certain actinomycin-producing organisms, including the Lindenbein culture of *S. chrysomallus* and a culture of *S. parvus*, were considered to belong to the *S. griseus* section. A detailed study of the actinomycin-producing organisms has recently been made by Solovieva and Dielova (1960).

Various other organisms belonging to the *S. griseus* section are able to form at least two other antibiotics. One of these, cycloheximide, is active only against fungi, and another, streptocin, is active against certain protozoan-like organisms. No detailed study has as yet been made of these strains in an effort to raise them to species status.

There are also those strains of *S. griseus* that produce no antibiotic at all, at least as far as one is able to detect by available methods.

The streptomycin-producing strains of *S. griseus* give rise readily to mutants. Two such mutants have been reported: One was a colorless form, producing no aerial mycelium, forming no streptomycin, and sensitive to this antibiotic; Dulaney *et al.* (1949) reported, however, on a colorless mutant that produced streptomycin. The other was a pigmented mutant, forming pink to vinaceous-colored substrate growth and an aerial mycelium typical of *S. griseus*; this mutant formed no streptomycin but gave rise to another antibiotic (rhodomycetin), which was not active against gram-negative bacteria. According to Kutzner (1960), this strain shows much similarity, on the basis of phage sensitivity and other properties, to *S. californicus*. A detailed study of degenera-

TABLE 21

Classification of actinomycin-producing organisms based upon utilization of rhamnose and raffinose (Ettlinger *et al.*, 1956)

Actinomycin	Species	Reference or origin of strain	Utilization of:	
			Rhamnose	Raffinose
+	*S. flavovirens*	Pridham and Gottlieb	+	+
+	*S. flaveolus*	Kurosawa	+	+
+	*S. antibioticus*	Pridham and Gottlieb	+	−
+	*S. antibioticus*	Burkholder *et al.*	+	−
X	*S. antibioticus*	orig. (Waksman)	+	−
X	*S. antibioticus*	NRRL (Raper)	+	−
C	*S. chrysomallus*	orig. (Lindenbein)	+	−
−	*S. flaveolus*	orig. (Waksman)	+	−
I	*S. parvullus*	orig. (Waksman)	+	−
X	*S. parvus*	NRRL (Benedict)	+	−
X		3 ETH strains	+	−
C		8 ETH strains	+	−
X		ETH 9001	−	−

TABLE 22

Grouping of actinomycin-producing organisms (Ettlinger *et al.*, 1956)

Group	Melanoid pigment	Spore color	Actinomycin complex	Authentic strains	ETH strains
I	+	cinereus	X	*S. antibioticus* (orig. and NRRL)	3
II	+	griseus	X		1
III	−	cinereus	I	*S. parvullus* (orig.)	1
			X		2
			C	*S. chrysomallus* (orig.)	17
IV	−	griseus	I		5
			X	*S. parvus* (NRRL)	

tion and regeneration of *S. griseus* was made by Williams and McCoy (1953).

Different varieties of *S. griseus* vary greatly in their cross-resistance and in their sensitivity to actinophages.

It must further be noted that high-yielding streptomycin strains can be obtained by irradiation, by growth in media containing increasing concentrations of streptomycin, and by strain selection.

Okami (1950a) examined 47 strains belonging to the *Griseus* series. Five of these were grisein-forming strains and four were pink pigmented forms. The streptomycin-producing strains grew in maltose-containing media with $NaNO_3$ as a source of nitrogen, but not in glucose, glycerol, or sucrose media. The grisein strains grew in media containing any of the four carbon compounds. The pink strains grew only in glycerol media (Table 23). The utilization of the carbohydrate was found to depend largely on the nitrogen source. In the presence of ammonium sulfate, the above differences disappeared. All strains utilized xylose, but not raffinose or rhamnose. Sensitivity to phage was said to be

strain specific, but not characteristic of streptomycin production. The use of streptomycin-resistant and streptomycin-dependent cultures of bacteria as test organisms for the differentiation of the various strains of *S. griseus* was considered as supplementary to the foregoing differentiation methods.

It is of particular interest, in this connection, to draw attention to the confusion that has arisen in some cases from Krassilnikov's attempt to change the name of the streptomycin-producing organism. Just as Waksman did previously, Krassilnikov came to the conclusion that there is a difference between the Krainsky and the Waksman and Curtis cultures of *A. griseus*. Although he, likewise, had no opportunity to compare Krainsky's original isolate with the streptomycin-producing organism, he attempted to draw conclusions on the basis of cultures that he isolated himself, and proposed that the name of the streptomycin-producing organism be changed to *Actinomyces globisporus streptomycini* (later changed to *A. streptomycini*). This suggested change was most unfortunate for several reasons: (a) a well described specific name, namely *Streptomyces griseus* Waksman and Henrici, was set aside merely for the sake of priority of a name of a culture (*A. griseus* Krainsky) which no one had ever seen and which was not available in any culture collection; (b) a name of an organism that had become recognized throughout the world because of its important physiological and biochemical properties, and especially because of its capacity to produce a highly important chemical substance, streptomycin, was changed to a trinomial merely because of the existence of an insufficiently described variety of an unknown culture.

The confusion thus became compounded by this attempt to change the name of the streptomycin-producing organism. We find, in addition to the two names suggested by Krassilnikov, the incorrect names *Actinomyces griseus* Waksman listed by Koreniako and Nikitina (Shorin, 1957), *Streptomyces griseus* Krassilnikov by Znamenskaia *et al.* (1957), *Actinomyces griseomycini* by Gause *et al.* (1957), and finally *Streptomyces globisporus streptomycini* by Severin and Gorskaia (1957). This confusion was fortunately limited to the literature published in Russian.

TABLE 23

Utilization of carbon sources by various strains of Streptomyces griseus *with nitrate as source of nitrogen* (Okami, 1950)

Carbon source	Streptomycin strain	Grisein strain	Pink-pigment strain
Glucose	−	+	−
Glycerol	−	+	+
Sucrose	−	+	−
Maltose	+	+	−

For the reasons presented here, Krassilnikov's modifications of the name *S. griseus*, with all the subsequent confusing names, cannot be accepted. In fact, the actual culture, *A. griseus* (Krainsky) *emend.* Krassilnikov (1949), belongs rather to the *Cinereus* series.

The logical name for the streptomycin-producing species remains *Streptomyces griseus* Waksman and Henrici.

Additional Organisms

Numerous other organisms belonging to the *Griseus* series have been described as species and as varieties. Some of the descriptions are incomplete; and it is, therefore, rather difficult to give them an exact position. This is true, for example, of the cultures described by Gause *et al.* (1957) under the name *A. rubiginosohelvolus* and some of the other species placed in the series *Helvolus*. See also Harada, 1959.

IX. Series *Hygroscopicus*

This series comprises organisms that form a white to gray aerial mycelium, with a

tendency to become dark gray; frequently black patches are produced in the mycelium, the whole often becoming black. The substrate growth is dark gray with a tendency to become moist, slimy, and finally changing to black. The species are melanin-negative, although on synthetic agar a brown to black soluble pigment may be produced.

Morphologically the species give rise to spiral-shaped sporophores. This series comprises a number of species, some of which are listed: *S. endus*, *S. hygroscopicus*, *S. limosus*, *S. nigrificans*, *S. platensis*, and *S. violaceoniger*.

X. Series *Scabies*

Characteristic Properties

a. Sporophores produce spirals.

b. Aerial mycelium light gray to dark gray.

c. Melanin-positive.

d. Some species are able to cause diseases of plants, notably scab of potatoes.

The melanin-producing capacity of certain actinomycetes, or their ability to form soluble brown to black pigments when grown in protein-containing media, was first recognized as a diagnostic characteristic by Rossi-Doria and Gasperini in 1891. Numerous cultures found capable of producing such pigments were isolated from different substrates, and designated as *Streptothrix chromogena*, *Actinomyces chromogenus*, or *A. chromogenes*. Gradually it came to be recognized that all these isolates represented not a single species but a large number of organisms, differing greatly in their morphological, physiological, and biochemical properties.

This was definitely established in 1900 by Beijerinck, who isolated two types of actinomycetes ("*Streptothrix*") represented abundantly in nature. "One of these that I have learned to recognize in the form of numerous varieties, I will designate as *Str. chromogena* Gasperini, since I believe that one such variety was available to the author of this name. The other species I designate as *Str. alba*." The first was characterized by the formation of a brown pigment in meat extract-gelatin media.

Neukirch (1902) demonstrated the presence in nature of two chromogenic types of actinomycetes. Krainsky (1914) described four chromogenic species, whereas Waksman and Curtis (1916) demonstrated the occurrence in soil of various other chromogenic types, "each with such well defined characters as to make it almost impossible to classify them as one species."

One of the most important series among the chromogenic actinomycetes is *S. scabies*, which at one time was designated as *S. chromogenus*. In addition to the members of the *Chromogenus* series as such, numerous other species now included in other groups also possess chromogenic properties, especially members of the *Lavendulae* and *Reticuli* series.

The soluble pigment produced by various organisms when grown on protein-containing media was found to belong to the melanin type. It frequently involved the tyrosinase reaction. The intensity of the pigment varies with the organism and with the medium. The formation of the melanin pigment is usually determined by growing the organisms on tyrosine-containing media.

Baldacci *et al.* (1953) did not recognize a "*Chromogenus*" series as such, although they listed one under "Cas-Gri." Gause *et al.* (1957) listed two series, one a "*Chromogenus*" proper, and the other "*Helvolus*," in which both pigment-producing and nonpigment-producing types are included.

The organisms belonging to *S. scabies* were at first believed to be primarily associated with scabbiness in white potatoes, sugar beets, and mangels. Only the typical chromogens were at first included in this series. They formed a brown to dark brown to black growth, a gray aerial mycelium, and a brown

to black soluble pigment on organic media. It must be conceded at once that not all organisms isolated from scabby potatoes or beets are able to produce a soluble brown pigment and certainly not all are capable of causing scabbiness in potatoes.

Millard and Burr (1926) isolated a number of cultures from scabby potatoes and beets. They proposed a key for the identification of the presumably potato-disease-producing actinomycetes (see Volume I, Chapter 18). The medium selected for this purpose (glycerol nitrate solution) could hardly be considered the most desirable substrate for bringing out the proper characters for a system of classification. Some of these cultures, notably *S. clavifer* and *S. fimbriatus*, definitely belong to the *Scabies* series.

Baldacci and Spalla (1956) suggested that the strain of *S. scabies* isolated by Millard and Burr be designated as *S. scabies* var. *anglica*. It is distinguished from the North American species first described by Thaxter as having a "gray substrate growth, a gray aerial mycelium and a yellow soluble pigment."

The possibility that different strains or races of *S. scabies* were responsible for the infection of potatoes and mangels has been fully recognized. No definite correlation has been found, however, between pathogenicity and cultural and other properties of the organism, although variants may differ from the parent culture in pathogenicity. High nitrogen content of the medium appeared to inhibit production of aerial mycelium in the parasitic strains but not in the saprophytes. Of the 20 isolates tested by Schaal (1944) on three different media, six did not produce any spirals but 14 did. These spirals were of both sinistrorse and dextrorse types.

Taylor and Decker (1947), in a study of 143 isolates obtained from scabby potatoes, beets, and radishes, used the following criteria for their classification: acid-fastness; starch hydrolysis; formation of dark brown surface ring on milk; acidification of milk; reduction of nitrate to nitrite; utilization of certain sugars, organic acids, and paraffin; gelatin liquefaction; pigment formation from tyrosine; and maximum growth temperature. The only true correlation between specific cultural properties and the ability to produce typical lesions of potato scab was obtained in the production of a dark brown ring of surface growth on milk.

The following species may be tentatively included in the *Scabies* series: *S. scabies*, *S. hawaiiensis*, and *S. galtieri*.

A number of other organisms isolated from potato tubers or directly from the soil were found capable of causing scab of potatoes and must be included in this series. This is true, for example, of *A. violaceochromogenes* described by Krassilnikov (1949), and of *A. chromofuscus* and *A. prunicolor* of Gause *et al.* (1957). Other closely related forms have been described, although pathogenicity tests were not made.

A number of forms that apparently have nothing to do with scab formation, but have the characteristic properties of the series may also be included.

XI. Series *Lavendulae*

Characteristic Properties

a. Sporophores straight or spiral-forming; spores oval, smooth surface.

b. Aerial mycelium colored lavender to pale blue.

c. Melanin-positive.

This is one of the true chromogenic series of the genus *Streptomyces*. Organisms belonging to the *Lavendulae* series are widely distributed in the soil and are represented there by a large number of species and varieties. Many of them are strongly antagonistic and are capable of forming various important antibiotics, such as streptothricin. Woodruff and McDaniel (1958) reported that 90 per cent of all the antibiotics produced by

actinomycetes are streptothricins; here belong various related compounds, such as streptin, streptolin, actinorubin, and antibiotic 136.

The most important species belonging to this series are *S. lavendulae* and *S. venezuelae*, organisms producing streptothricin and chloramphenicol respectively.

S. lavendulae comprises organisms extremely variable in nature. Many of them give rise, on cultivation, to different variants or mutants. Some of these variants produce a blue diffusible pigment on glucose-peptone agar; others form a brown pigment. The substrate mycelium of the blue pigment-forming variants is pale blue, with scattered, small pinpoint areas of deep blue. Upon complete sporulation, the substrate growth becomes covered with the characteristic lavender-colored aerial mycelium; occasional sunken areas have a slightly bluish tinge; the reverse of the substrate growth is cream-colored except for the small blue spots. Other variants produce a colorless to cream-colored substrate growth free of any blue pigment whatsoever; a brown diffusible pigment appears later, and the growth becomes covered with thick lavender-colored mycelium. The two types of variants are stable in nature. Some variants may lose the capacity to produce aerial mycelium.

S. venezuelae, as well, gives rise to a number of variants. Two strains were isolated and found to be similar to *S. lavendulae* in their cultural and physiological properties, although they differed in their ability to utilize various carbohydrates. *Streptomyces venezuelae* utilizes arabinose, rhamnose, xylose, lactose, and fructose; *S. lavendulae* has either no effect or only a limited effect upon these carbohydrates. The former also differs from the latter in its sensitivity to actinophage and in various serological reactions.

Streptomyces venezuelae was described as having a thin-walled substrate mycelium, colorless, hyaline, monopodially branched, the hyphae varying in diameter from 0.9 to 1.8 μ and the branches growing to about 150 μ in length. The aerial mycelium appears lavender under the microscope, thick-walled, generally not much branched, straight or slightly and irregularly curved, not forming spirals, individual hyphae arising frequently from the primary mycelium at the surface of the substrate. The color of the colonies, when viewed on agar without magnification, is gray to light tan or pink, but not lavender. The upper portions of the aerial hyphae divide into chains of spores. These are oval to oblong 0.4 to 0.9 by 0.7 to 1.6 μ. Individual spores are colorless at maturity, but in mass appear tan to gray when viewed without magnification.

Okami (1956) made a comparative study of the organisms commonly included in the *Lavendulae* series on the basis of the color of the aerial mycelium and certain other characteristics. He found that eight cultures, notably the streptothricin-producing forms, possessed the following properties which he considered as standard for the series.

a. Aerial mycelium pink-lavender color when grown on yeast extract-glucose agar.
b. Brown pigment when grown on yeast extract-glucose agar.
c. Very sensitive to chlortetracycline and chloramphenicol; relatively sensitive to streptomycin; relatively resistant to neomycin; and resistant to streptothricin.
d. Utilize: glucose, galactose, maltose, mannose. Do not utilize: arabinose, fructose, lactose, mannitol, raffinose, rhamnose, sucrose, xylose.
e. They show certain growth-inhibiting effects (Tables 24 and 25).

On continued cultivation for 40 years on artificial media, the original 1915 isolate of *S. lavendulae* (No. 3330) lost many of its characteristic properties:

1. It no longer produced any aerial mycelium.

2. It did not form any dark brown pigment.
3. It was now able to utilize fructose, mannitol, rhamnose, xylose.
4. It showed no or very weak antagonistic action.

On the basis of the above properties, Okami divided the *S. lavendulae* series into 10 subgroups (Table 26).

TABLE 24

Antibacterial activity of different strains of Streptomyces lavendulae (Okami, 1956)

S. lavendulae strain no.	Inhibition zone, test bacteria		
	E. coli	*Staph. aureus*	*B. cereus*
	mm	mm	mm
3330	0	0	0
3440-S	17	16	3
3440-14	6	6	2
3516	18	17	2
3516-W	10	10	2
3530	16	16	2
3531	0	0	0
3532	27	23	18
3542	18	17	11
3543	11	18	6
3568	0	0	0
3445	0	0	0
3465	2	4	5
3544	11	9	3
3555	33	27	21

Baldacci considered *S. lavendulae* as a distinct group. Krassilnikov, however, looked upon these organisms as members of the "*chromogenus*" group. Gause *et al.* created a series under the name "*lavendulae-roseus*" and subdivided it into three subgroups, based upon the formation of a soluble pigment in organic media: (1) The first included cultures that formed no soluble pigment, such as *S. virginiae*; (2) The second included *S. venezuelae*, *S. lavendulae*, and *S. circulatus*, as well as a variety of others, all of which formed a brown to black pigment in organic media; (3) The third produced a yellow pigment. As the requirements for the group *S. lavendulae* presented above indicate, neither the first nor the third of these subgroups belong to this group.

Sánchez-Marroquín (1958) found that three related species of *Streptomyces* producing a pink to lavender aerial mycelium on synthetic media could be distinguished from one another as follows: (a) *S. fradiae* produces spirals on short branches; (b) *S. lavendulae* forms spirals at the tip of long straight branches; (c) *S. venezuelae* forms no spirals, but only straight sporophores; the first is nonchromogenic and the last two are chromogenic. The *Lavendulae* series was

TABLE 25

Effect of composition of medium on reciprocal antagonism between different strains of Streptomyces lavendulae (Okami, 1956)

Strain number	Inhibition zone of *S. lavendulae* 3516				Inhibition zone of *S. lavendulae* 3440-S			
	YGA	Agar media*		Soya	YDA	Agar media		Soya
		ASP	Starch			ASP	Starch	
	mm	mm	mm	mm	mm	mm	mm	mm
3330	4.0	5.0	7.0	4.0	3.0	0	2.0	3.0
3440-S	17.0	15.0	20.0	15.0	0	0	0	0
3440-14	17.0	4.0	18.0	10.0	0	0	0	0
3516	0	0.5	0	0	0	0	0	0
3516-W	0	2.0	3.0	1.0	0	0	0	0
3530	0	1.0	1.0	1.0	0	0	0	0
3531	12.0	1.0	5.0	8.0	0	0	0	0
3532	16.0	10.0	2.0	12.0	7.0	0	10.0	10.0

* YGA = yeast extract-glucose; ASP = asparagine-glucose.

TABLE 26

Classification of the lavendulae *series into subgroups* (Okami, 1956)

Subgroup	Culture No.	Characteristics
A		Standard *S. lavendulae*
	3440–8	a. Aerial mycelium with pink-lavender color on YDA
	3440–14	b. Brown pigment on YDA
	3516	c. Very sensitive to chlortetracycline and chloramphenicol, relatively
	3516–W	sensitive to streptomycin, relatively resistant to neomycin, resistant to
	3530	streptothricin
	3542	d. Utilizes: galactose, maltose, mannose
	3544	Does not utilize arabinose, fructose, lactose, mannitol, raffinose, rham-
	3555	nose, sucrose, xylose
		e. Shows certain inhibiting effects
B	3330	a. No aerial mycelium
		b. No dark brown pigment
		d. Utilizes fructose, rhamnose, mannitol, xylose
		e. Antagonistic action none or very weak
C	3531	c. Relatively resistant to chloramphenicol
D	3532	c. Relatively resistant to chlortetracycline
		e. Antagonistic action none or weak
E	3543	b. No dark brown pigment
		c. Relatively resistant to chloramphenicol
		d. Utilizes arabinose, fructose, lactose, mannitol, raffinose, rhamnose, su-
		crose, xylose
		e. Antagonistic action none or weak
F	3568	d. Utilizes arabinose
		e. Antagonistic action none or weak
G	3465	b. No dark brown pigment
		e. Antagonistic action none or weak
H	3555	c. Relatively sensitive to streptothricin VI
		d. Utilizes fructose
		e. Antagonistic action none or weak
I	3625	c. Resistant to chloramphenicol
	3534	d. Utilizes arabinose, rhamnose, xylose
	3534–A	e. Antagonistic action none or weak
J	3651*	d. Does not utilize mannose
	3652*	

* *S. virginiae*

said to include not only *S. lavendulae* but also *S. cinnamonensis* and *S. virginiae*.

To indicate the confusion in classifying members of the *Lavendulae* series, it is sufficient to cite the work of Kuchayeva (1958). She collected 22 cultures, freshly isolated or obtained from different laboratories and believed to belong to this group.

Eight of the cultures produced straight sporophores and 14 formed spiral-shaped sporophores. The color of the aerial mycelium of the substrate growth varied from yellow to reddish-brown. Some produced a melanin pigment and others did not, thus automatically excluding the last as members of the *Lavendulae* series. They varied greatly in their antagonistic properties, some inhibiting the growth of all bacteria and fungi tested, and others having no effect either on gram-negative bacteria or on certain fungi. The following antibiotics were listed as products of the *Lavendulae* series, thus suggesting the possible specific differences in the series: streptothricin, streptin, antismegmatis factor, antibiotic 136, lavendulin, actinorubin, pleocidin, ehrlichin, actithiazic acid, antibiotic MD 2428, and grasseriomycin.

XII. Series *Erythrochromogenes*

This is a large melanin-positive series of organisms. The aerial mycelium is usually white with a yellowish or brownish shade to gray with a bluish or greenish shade; it is often reddish to brown. The substrate growth is colorless to orange to red or even brown to black. Certain species placed in this group often produce a greenish-yellow or reddish-brown to almost black soluble pigment on synthetic media. The sporophores are straight or produce spirals.

This melanin-positive series comprises a large number of species, such as represented by the following: *S. erythrochromogenes*, *S. bobiliae*, and *S. cinereoruber*.

XIII. Series *Viridochromogenes*

This melanin-positive series of organisms is characterized by an ash-gray to greenish to olive-green to bluish aerial mycelium. The substrate growth is cream-colored to brown to greenish to black. Soluble pigment on inorganic media is yellowish to greenish to black. On organic media, soluble pigment is brown to deep brown to olive or purple or black.

The sporophores form spirals.

This series is widely represented in nature by a number of species, namely: *S. viridochromogenes*, *S. chartreusis*, and *S. cyaneus*.

XIV. Series *Cinnamomeus*

Characteristic Properties

a. Sporophores produced in verticils.

b. Aerial mycelium white, yellow, or pinkish.

c. Substrate growth yellowish to pinkish.

d. Melanin-negative.

This series is characterized by the formation of verticil-bearing sporophores. The verticils are both primary and secondary. Spirals usually are not produced, although occasionally some spirals are formed. The species within this series are melanin-negative, although a purplish pigment may be produced on certain organic media. The aerial mycelium is white to pinkish to cinnamon-colored. The substrate growth is yellowish to brown to pinkish.

The species included in this series can be listed here: *S. hachijoensis*, *S. fervens*, and *S. cinnamomeus*.

XV. Series *Reticuli*

Characteristic Properties

a. Sporophores produced in verticils, straight or spiral-shaped, on the primary or secondary aerial hyphae.

b. Aerial mycelium white to gray.

c. Melanin-positive.

This is one of the two series within the genus *Streptomyces* which are differentiated from the other members of the genus primarily by their morphology. Species within this series are characterized by the radial arrangement of the sporophores, whereby three or more branches originate from a

node, thus forming a "verticil," frequently referred to as a "whorl." Very often the sporophores may be branched toward the end of the sporulating-hyphae, giving the appearance of a "broom shaped branch," or may give rise to the formation of a "cluster," which is to be distinguished from a typical verticil of sporophores. The verticils may be primary or secondary in nature. Among the variations of a verticil is the formation of tufts, when straight branches are grouped together on the aerial hyphae.

The formation and nature of the verticils may be changed with the composition of the medium, a phenomenon first reported by Waksman and Curtis (1916) and more recently by Nakazawa (1955) and Shinobu (1955a). Both primary and secondary verticils may be formed in the same culture.

Baldacci (1953) did not recognize this important series at all. He gave series recognition to one of its members, *S. rubrireticuli*, merely on the basis of its pigmentation. Gause *et al.* (1957), following this example, included in the "*ruber*" series one "tuft-forming" organism, also on the basis of its pigmentation. Later, however, Baldacci (1958) suggested separation of the verticil-producing organisms into a separate genus, *Streptoverticillium*, as shown elsewhere (Chapter 4, Volume I).

Various investigators have used morphology as a basis for series separation of the genus *Streptomyces*. It is sufficient to list here three of them.

Shinobu (1955a) proposed division of the genus as follows:

1. Monopodial branching. This section has been divided on the basis of spiral formation.
2. Verticil formation:
   a. *Nitella* type. Typical radial branches almost equal in length. No spirals formed. *S. reticuli* is given as a typical representative.
   b. *Anitella* type. Radial branches differ from each other. Spirals formed without proper radial symmetry. *S. viridochromogenes* is representative.

The *S. reticuli* subgroup was further divided into:

A. Verticils only primary. Sporophores straight.
   Streptomyces verticillatus
   Streptomyces hiroshimensis
B. Verticils both primary and secondary. Sporophores form spirals.
   I. Colorless to brownish growth on synthetic media.
      1. Good growth on protein media. Spores spherical, oval.
         Streptomyces reticuli
         Streptomyces albireticuli
      2. Poor growth on protein media. Spores cylindrical.
         Streptomyces circulatus
   II. Growth on synthetic media pink to red.
      Streptomyces reticuloruber
      Streptomyces griseocarneus
   III. Growth on synthetic media greenish.
      Streptomyces verticilloviridans

Solovieva *et al.* (1957) made a study of cultures belonging to the *S. reticuli* series and isolated from Pamir soils. These cultures were divided into two subgroups:

I. *S. verticillatus*, with straight sporophores (primary and secondary).
II. *S. reticuli*, with spiral-shaped sporophores.

These subgroups showed very little difference in their physiological and biochemical properties. Only one strain, *S. rubrireticuli*, showed some differences (weaker gelatin liquefaction, strong nitrate reduction, weak growth on cellulose). From an antibiotic point of view, however, there was a marked difference; members of the first subgroup showed strong antifungal activity, whereas subgroup II gave weaker activity or none.

Pridham *et al.* (1958) divided the verticil-forming series into four morphological subgroups:

1. Monoverticillate, no spirals.
2. Monoverticillate, with spirals.

3. Biverticillate, no spirals.
4. Biverticillate, with spirals.

The following species were included in this series: *S. reticuli*, *S. netropsis*, *S. thioluteus*, *S. griseocarneus*, and *S. verticillatus*.

Unfortunately, some cultures show both straight and spiral-shaped sporophores. Nomi (1960) criticized the above system; he could not accept the spiral-producing forms among the true verticil types. He considered the group to consist of the typical "biverticils" comprising both primary and secondary elements; the atypical "monoverticils" comprising primary verticils, mixed verticils, and sometimes more compound polyverticils. He included the following species: *S. reticuli*, *S. hiroshimensis*, *S. albireticuli*, *S. echimensis*, *S. griseocarneus*, *S. thioluteus*, *S. salmonicida*, and *S. netropsis*.

Series *Thermophilus* is discussed in Chapter 11, among the thermophilic actinomycetes.

Other Possible Series

In addition to these 16 series of the genus *Streptomyces*, other series could be suggested, based either on the color of the aerial mycelium or of the substrate growth; ecological or physiological criteria have also been proposed for series characterization. Certain groups, such as the thermophilic forms belonging to the genus *Streptomyces* are included in a special series (*Thermophilus*), and are discussed elsewhere (Chapter 10), since they are definitely related, because of their close ecological and physiological relationships, to the other thermophilic genera.

Chapter 7

Classification of *Streptomyces* Species

The difficulties encountered by an inexperienced investigator in identifying a freshly isolated culture of a *Streptomyces* with previously described species have been brought out in the preceding three chapters. No wonder an inexperienced worker soon becomes discouraged and takes the easy path of creating a new species or variety for every newly isolated culture. This is especially true if such a culture produces an antibiotic not previously described or an apparently different form of a known antibiotic that he is anxious to patent or on which he wishes to establish priority. Among the significant factors that have contributed to this rash of "new" species are:

1. Inadequate description of previously described species with which comparisons are made.

2. Overlapping of the morphological and cultural characteristics of strains or species previously described.

3. Variations in composition of the media used in describing species.

4. Failure to recognize natural variability of different strains that might be included in a single species.

5. Idiosyncrasies of the particular investigator, and his tendency to be either a "lumper" or a "splitter."

In spite of these discouraging aspects of the problem of identification of particular organisms, classification and characterization of *Streptomyces* species have recently made considerable progress. Though various criticisms have been directed toward it, the system of classification of actinomycetes used in the seven editions of Bergey's Manual still appears to be the most logical and most workable, except for certain modifications that are now desirable. In every new edition, advantage has been taken of the accumulated information to modify this system of classification, especially of the genus *Streptomyces*. In this treatise, a complete rearrangement has been made in classifying the species included in the genus *Streptomyces*, as compared with the last (seventh) edition of Bergey's Manual.

The thermophilic species of *Streptomyces* have been placed in a separate series *Thermophilus* and transferred to Chapter 11, in which all the thermophilic actinomycetes are included. Species of *Streptomyces* isolated from animal and plant infections, especially those for which pathogenicity has not been established, have been distributed throughout the genus, thus removing the need for a major separation of these species into saprophytic *versus* parasitic forms. These properties are now given only secondary consideration in characterizing species within the genus. It has further been recognized and emphasized, time and again, that it is most desirable to utilize morphological properties in defining and characterizing species of *Streptomyces*, though in some instances this could not be done with any degree of assurance.

New genera have been created, once certain well-defined morphological and physiological properties suggested its advisability.

Whether this practice should be extended and recognition thus be given to the capacity to form sclerotia or to the ability to form verticils by certain *Streptomyces* species, thus placing them in separate genera, remains to be determined. The author's suggestion of many years ago that the structure of the sporophores (straight *versus* spiral-forming, closed *versus* open spirals, tuft- and verticil-producing) be used in characterizing certain species or species-groups is gaining wider recognition, though some investigators do not consider this a sufficiently constant property for the major subdivision of the genus and suggest that it be left for secondary characterization. The shape and size of the spores appear to be less significant properties, although the surface of the spores, as detected by the electron microscope, has been gaining approval.

Among the most annoying characteristics of the genus *Streptomyces* are: (a) the loss of capacity by certain species to produce aerial mycelium, and (b) the overlapping property on the part of certain species of *Nocardia* to produce an aerial mycelium that cannot be differentiated from that of *Streptomyces*. It is true, however, that cultures of *Streptomyces*, even if they have lost the capacity to produce aerial mycelium, can still be recognized by the structure of their substrate mycelium and by certain cultural and physiological properties. The latter include the nature of their soluble pigments, their ability to liquefy gelatin, hydrolyze starch, invert sucrose, and coagulate and peptonize milk. Some strains that have lost the capacity to produce aerial mycelium may regain this property if they are grown in sterile soil or in special soil media, or are subjected to other special treatments.

The growth of cultures of *Streptomyces* that have lost the capacity to produce aerial mycelium is often colorless, though sometimes pigmented; it is smooth or lichenoid, leathery, compact, with a shiny surface. Some produce a soluble brown pigment. Some are able to form antibiotics. On the assumption that such cultures, because they do not form aerial spores, should be considered as sterile, Krassilnikov designates them as trinomials with a third component of the name "sterilis." This is analogous to *Fungus sterilis* among the fungi. Certain such species are included in the present classification; others may be considered as typical nocardias and have been transferred to that genus.

Characterization of *Streptomyces* Species

Among the properties to receive major consideration in describing individual species are the following:

1. *Morphological properties:* These include formation and nature of substrate (vegetative) growth and of aerial mycelium, manner of sporulation (spiral formation, verticil formation), nature and surface of spores.

2. *Cultural characteristics.* These comprise color and color changes of substrate growth and of aerial mycelium, and formation of soluble pigments on synthetic and organic media. The most significant of these pigments are the melanins or melanoids produced in media containing tyrosine or proteins and peptones. The brown to black pigments produced in such media by certain species of *Streptomyces* are said to designate melanin-positive as opposed to melanin-negative reactions, or tyrosinase-positive *versus* tyrosinase-negative reactions. In view of the fact, however, that few of the older investigators tested this reaction in tyrosine–free media, it is more desirable to use the designations chromogenic (melanin +) or nonchromogenic (melanin −). In this case, chromogenicity refers specifically to the formation of brown to black pigments on protein-containing media.

3. *Physiological and biochemical properties.* These include: proteolytic activities, such as

gelatin liquefaction, coagulation and peptonization of milk, and hemolysis of blood; utilization of carbon compounds; antagonistic properties and formation of antibiotics; effect of temperature, aeration, and reaction upon growth; formation of specific enzymes, such as oxidase, lipase, invertase, diastase, mannase and protease; reduction of nitrate, and formation of H_2S. There is no sharp line of demarcation between cultural and physiological properties, on the one hand, and between physiological and biochemical activities, on the other.

4. *Ecology.* The ability of the organism to cause animal or plant diseases, and its occurrence in a natural environment are important characteristics.

5. *Supplementary characteristics.* Additional characteristics that may be utilized for descriptive purposes include: (a) serological reactions, (b) phage sensitivity, and (c) sensitivity to specific antibiotics.

In describing the various species, one has to depend frequently upon the information supplied in published reports, since type cultures often are not available. Where the desired information is lacking or where the description, for various reasons, is inadequate, the species may be placed in the list of incompletely described forms.

Recently there has been a tendency to overlook earlier described species, and to emphasize and often give new names to newly isolated cultures. One cannot condemn this tendency too strongly. While, in most cases, it is difficult to establish synonymy because of a lack of type cultures or a possible change in such cultures upon prolonged cultivation on artificial media, every effort must be made to give credit to the earlier investigator. One has no patience, therefore, with those attempts to set aside, willfully or unwillfully, older descriptions or to consider such organisms as varieties of newly isolated and newly named cultures.

When a culture is freshly isolated, a study should be made both of its position in a particular group in the genus and of its classification as a species. Only by a combination of properties described under both can one determine the identity of the new culture. Obviously, no conclusions should be drawn that such a culture represents a new species merely on the basis of certain superficial observations, such as a delay in coagulation and peptonization of milk or in liquefaction of gelatin, or because of a difference in the intensity of coloration of the potato plug, or in the shade of pigmentation of growth on a particular medium, or even in the degree of curvature of the sporophores. Any attempt to create new species on the basis of such minor variations must be considered both unscientific and confusing. The tests must be repeated again and again to confirm the recorded observations.

The need for a knowledge of the exact composition of the media used for descriptive purposes can hardly be overemphasized. Some species show their most characteristic property upon only one particular medium, and unless such a medium is used, the properties of a new isolate can easily be overlooked. *S. fradiae*, for example, shows the characteristic color (seashell-pink) of its aerial mycelium upon potato-starch agar. Certain synthetic agar media, notably nitrate-sucrose, glucose-asparagine, and calcium malate, are among those which alone bring out characteristic properties of certain other species. Growth on potato is quite characteristic, although the variety of the potato, the manner of crop fertilization, and other factors may influence the nature of the growth of the organism and pigment formed.

In view of the great interest at the present time in the screening programs for antibiotics, when literally many thousands of cultures are being isolated and tested, there is naturally a tendency on the part of some investigators to consider the ability of a particular culture to form a specific anti-

biotic as its major characteristic property. But this capacity is often a strain rather than a species characteristic. In view of the mutational possibilities of such cultures and of the marked effect of composition of medium and environmental factors upon the qualitative nature and quantitative yield of the antibiotic, one must consider such a property, at best, as only a secondary characteristic and avoid assigning to it an important role in creating new species.

Shinobu (1958b) emphasized again that only synthetic media should be used for the study of the sporulation of the aerial mycelium, notably spiral formation, in *Streptomyces*. In most species, the curvature of the spiral is sinistrorse (counterclockwise); in a few, dextrorse (clockwise). The diameter of the spiral varies from 1.5 to 8.0 μ and is not a characteristic property, although it is fixed for some species. As pointed out previously (Chapter 4), three morphological groups were recognized: (1) Those forming straight or wavy aerial mycelium, (2) Spiral-forming types, and (3) Verticil-forming types.

Other criteria have been suggested. Some of these may be utilized for supplementary information in describing species and varieties. A Subcommittee on Actinomycetes of the Society of American Bacteriologists (Gottlieb, 1960) gave careful consideration to the various criteria used in describing and characterizing species of *Streptomyces*. They came to the conclusion that morphology is to be considered as one of the more important criteria. Color of the aerial mycelium and carbon utilization are also important. Supplementary characteristics are provided by the production of H_2S, reduction of nitrate, and gelatin liquefaction. Such criteria as color of substrate growth and nitrogen utilization were not considered of sufficient significance in describing new species.

Many species, including a number of newly isolated forms, have been placed in Chapter 13 as incompletely described. Others have been listed as synonyms. Care has been taken to avoid the creation of many new varieties, unless it has been fully established that such varieties have a sound morphological or cultural basis. Whether these varieties should be raised to the status of species remains to be determined by further study. Any such attempt would automatically lead to the temptation to create new species out of mutants, which are unfortunately altogether too common, whether naturally occurring or artificially created. For the time being, they may still be considered as varieties.

Classification of Genus *Streptomyces*

In proposing the present system of classification of the genus *Streptomyces*, the following properties have been given the greatest consideration:

1. Morphology of sporulating bodies; size, shape, and surface of spores.
2. Color of aerial mycelium and of substrate growth.
3. Color of growth.
4. Formation of soluble chromogenic or melanoid pigments in proteinaceous media. This property is used, together with micromorphology, for the major subdivisions of the genus.
5. Formation of soluble pigments in synthetic media.
6. Certain biochemical properties, notably proteolysis, starch hydrolysis, nitrate reduction, formation of H_2S, utilization of carbon sources, and formation of specific antibiotics.

This system is a modification of the one originally used by Waksman and Curtis in 1916, variously changed in subsequent years by Waksman and by Jensen, and used in modified forms in the various editions of Bergey's Manual of Determinative Bacteriology.

A. Sporophores straight, wavy, or spiral-shaped; no verticils.

I. Proteinaceous media are not pigmented deep brown or black; melanin-negative. Soluble pigment on various media is faint brown, pink, red, purple, yellow, blue, or absent.
  1. Soluble pigment only faint yellow or faint brown.
    a. Aerial mycelium white, abundant.
      a^1. Sporophores produce spirals.
        a^2. Occurs in soil and in certain other natural substrates.
          15. *Streptomyces albus*
        b^2. Occurs in the sea.
          148. *Streptomyces marinus*
      b^1. Sporophores straight.
        a^2. Sporophores produce broom-shaped clusters.
          102. *Streptomyces globisporus*
        b^2. No clusters produced.
          a^3. Nonproteolytic.
            27. *Streptomyces autotrophicus*
          b^3. Weakly proteolytic.
            179. *Streptomyces orientalis*
          c^3. Strongly proteolytic.
            129. *Streptomyces kimberi*
    b. Aerial mycelium, white, scant.
      250. *Streptomyces willmorei*
    c. Aerial mycelium white to light gray.
      a^1. Sporophores produce compact spirals.
        a^2. Growth colorless.
          18. *Streptomyces annulatus*
        b^2. Growth colorless to yellowish.
          92. *Streptomyces fungicidicus*
        c^2. Growth brown to orange-brown.
          21. *Streptomyces arenae*
        d^2. No growth on sucrose nitrate agar.
          22. *Streptomyces argenteolus*
      b^1. Sporophores form loose spirals.
        40. *Streptomyces calvus*
      c^1. Sporophores straight or wavy.
        a^2. Growth on sucrose nitrate agar yellowish-brown.
          2. *Streptomyces albaraviensis*
        b^2. Growth gray to greenish.
          60. *Streptomyces coroniformis*
        c^2. Growth yellowish-white; utilizes paraffin and rubber.
          68. *Streptomyces elasticus*
    d. Aerial mycelium white to mouse-gray; spores bluish-gray.
      a^1. Sporophores produce compact spirals.
        227. *Streptomyces spheroides*
      b^1. Sporophores in clusters; a few compact spirals.
        45. *Streptomyces catenulae*
      c^1. Sporophores produce open corkscrew spirals.
        a^2. Soluble pigment yellow.
          a^3. Growth on synthetic media cream-colored.
            167. *Streptomyces niveus*
          b^3. Growth on synthetic media yellow.
            143. *Streptomyces macrosporeus*
        b^2. Soluble pigment tan to brown.
          35. *Streptomyces caelestis*
      d^1. Sporophores straight.
        111. *Streptomyces griseolus*
    e. Aerial mycelium white to gray, covered with dark humid stains or guttation drops.
      a^1. Sporophores form spirals.
        a^2. Growth buff to olive-colored.
          189. *Streptomyces platensis*
        b^2. Growth colorless.
          123. *Streptomyces humidus*
      b^1. Sporophores straight.
        a^2. Growth on potato cream-colored.
          61. *Streptomyces craterifer*
        b^2. Growth on potato slimy to black.
          235. *Streptomyces tumuli*
    f. Aerial mycelium green.
      a^1. Growth green.
        196. *Streptomyces prasinus*
      b^1. Growth colorless.
        121. *Streptomyces hirsutus*
      c^1. Growth red.
        195. *Streptomyces prasinopilosus*
    g. Aerial mycelium limited, produced late; white with tinge of gray.
      97. *Streptomyces gardneri*
  2. Soluble pigment blue or purple.

a. Aerial mycelium white.
63. *Streptomyces cyanoflavus*
b. Aerial mycelium white to gray.
a¹. Soluble pigment produced only on potato and certain other media; pigment changes to red in an acid and to green in an alkaline reaction.
58. *Streptomyces coelicolor*
b¹. Pigment produced on all media, red in an acid and blue in an alkaline reaction.
240. *Streptomyces violaceoruber*
c¹. Pigment at first yellow-red, changing to blue or bluish-green.
190. *Streptomyces pluricolor*
d¹. Pigment purple.
171. *Streptomyces novaccaesareae*
e¹. Soluble pigment bluish to black.
239. *Streptomyces violaceoniger*
c. Aerial mycelium blue.
a¹. No spirals formed.
36. *Streptomyces caeruleus*
b¹. Open spirals.
241. *Streptomyces violaceus*
3. Pigment at first green, becoming brown.
a. Aerial mycelium usually absent.
237. *Streptomyces verne*
b. Aerial mycelium white.
221. *Streptomyces sampsonii*
c. Aerial mycelium brownish-white to brownish-gray.
187. *Streptomyces phaeoviridis*
d. Aerial mycelium dark gray, olive-colored, or grayish-green; sporophores produce spirals.
244. *Streptomyces viridans*
4. Pigment yellow to golden yellow.
a. Growth green to greenish-yellow.
a¹. Aerial mycelium weakly developed; white or pale yellow.
242. *Streptomyces virgatus*
b¹. Aerial mycelium gray to dark gray.
125. *Streptomyces intermedius*
c¹. Aerial mycelium scant, white.
144. *Streptomyces maculatus*
d¹. Aerial mycelium cinnamon-colored.
158. *Streptomyces murinus*
b. Growth green to dark green.
14. *Streptomyces alboviridis*
c. Growth sulfur-yellow.
a¹. Aerial mycelium white to pinkish.
81. *Streptomyces flaveolus*
b¹. Aerial mycelium light yellow.
182. *Streptomyces parvus*
c¹. Aerial mycelium white to gray to reddish-gray.
251. *Streptomyces xanthophaeus*
d¹. Aerial mycelium light gray.
48. *Streptomyces cellulosae*
e¹. Aerial mycelium ash-gray.
181. *Streptomyces parvullus*
f¹. Aerial mycelium yellowish-green to sulfur-yellow.
230. *Streptomyces sulphureus*
d. Growth carmine red, reddish-brown to orange-colored to cinnamon-drab.
a¹. Aerial mycelium chalk-white.
166. *Streptomyces niveoruber*
b¹. Aerial mycelium white to gray.
a². No aerial mycelium on potato.
208. *Streptomyces rimosus*
b². Aerial mycelium on potato white to gray to black.
25. *Streptomyces aureofaciens*
Closely related form.
222. *Streptomyces sayamaensis*
c². Aerial mycelium on potato olive-buff.
57. *Streptomyces clavifer*
c¹. Aerial mycelium grayish-brown.
220. *Streptomyces sahachiroi*
d¹. Aerial mycelium yellowish-gray.
110. *Streptomyces griseoflavus*
e. Growth cream-colored to brown.
a¹. Rapid liquefaction of gelatin.
7. *Streptomyces albidoflavus*

b¹. Gelatin slowly liquefied.
50. *Streptomyces chibaensis*

5. Soluble pigment yellowish to yellow-green.
a. Aerial mycelium white.
51. *Streptomyces chrysomallus*
b. Aerial mycelium white to yellow.
a¹. Growth yellowish to green.
134. *Streptomyces lieskei*
b¹. Growth yellow, becoming black.
135. *Streptomyces limosus*
c. Aerial mycelium gray.
a¹. Growth on sucrose nitrate agar yellowish-green.
85. *Streptomyces flavovirens*
b¹. Growth on sucrose nitrate agar yellow.
47. *Streptomyces cellulosflavus*

6. Soluble pigment yellowish-brown to reddish-brown.
a. Growth cream-colored.
a¹. Sporophores flexible and hooked.
112. *Streptomyces griseoluteus*
b¹. Sporophores produced in clusters.
165. *Streptomyces nitrosporeus*
b. Growth has rosy tinge.
204. *Streptomyces ramnaii*
c. Growth yellowish.
17. *Streptomyces ambofaciens*
d. Growth has reddish tone.
191. *Streptomyces pluricolorescens*
e. Growth becoming red.
a¹. Aerial mycelium white.
70. *Streptomyces erythraeus*
b¹. Aerial mycelium white to gray with greenish tinge.
205. *Streptomyces ramulosus*
c¹. Aerial mycelium mouse-gray to drab.
83. *Streptomyces flavogriseus*
d¹. Aerial mycelium white to gray to olive-buff.
224. *Streptomyces setonii*

7. Soluble pigment in synthetic media brown.
a. Growth coral-red.
24. *Streptomyces aurantiacus*
b. Growth yellow.
32. *Streptomyces bottropensis*
c. Growth yellowish-brown
3. *Streptomyces achromogenes*
d. Growth brown to purplish.
16. *Streptomyces althioticus*
e. Growth black.
162. *Streptomyces niger*.

8. Soluble pigment on potato plug brown to brownish-red to reddish-purple.
a. Growth on potato greenish-colored.
a¹. Spirals formed.
65. *Streptomyces diastaticus*
b¹. No spirals.
142. *Streptomyces lydicus*
b. Growth on potato gray; no spirals formed.
42. *Streptomyces canescus*
c. Growth on potato yellowish-colored.
a¹. Aerial mycelium white.
87. *Streptomyces flocculus*
b¹. Aerial mycelium gray to yellowish.
a². Growth cream-colored.
80. *Streptomyces fimicarius*
b². Growth yellow-brown.
75. *Streptomyces felleus*
d. Growth on potato yellow turning white.
147. *Streptomyces marinolimosus*
e. Growth on potato pink to reddish-purple.
a¹. Sporophores produce spirals.
a². Aerial mycelium cinnamon to drab-gray.
170. *Streptomyces noursei*
b². Aerial mycelium gray.
11. *Streptomyces albogriseolus*
b¹. Sporophores both straight and spiral-forming.
228. *Streptomyces spiralis*
c¹. Growth on various media yellow-orange to brown.
89. *Streptomyces fragilis*

9. Soluble pigment on synthetic agar brown to black.
   a. Growth on potato gray to brown.
      74. *Streptomyces exfoliatus*
   b. Growth on potato greenish to black.
      100. *Streptomyces gelaticus*
   c. Aerial mycelium pigmented green.
      101. *Streptomyces glaucus*
10. No soluble pigment on synthetic media.
   a. Growth yellowish-brown.
      160. *Streptomyces narbonensis*
   b. Growth yellowish to pink to black.
      a^1. Aerial mycelium abundant, gray.
         23. *Streptomyces armillatus*
      b^1. Aerial mycelium white, with pinkish to orange tinge on certain media.
         77. *Streptomyces filamentosus*
      c^1. Aerial mycelium white to yellow.
         127. *Streptomyces kanamyceticus*
      d^1. Aerial mycelium white to pink.
         145. *Streptomyces madurae*
      e^1. Aerial mycelium scant, white.
         183. *Streptomyces pelletieri*
      f^1. Aerial mycelium white-gray to black.
         225. *Streptomyces somaliensis*
      g^1. Aerial mycelium black.
         180. *Streptomyces paraguayensis*
   c. Growth yellowish to orange.
      a^1. Aerial mycelium white to rose-colored.
         213. *Streptomyces roseoflavus*
      b^1. Aerial mycelium white.
         201. *Streptomyces putrificus*
      c^1. Aerial mycelium scant, white to grayish-brown.
         90. *Streptomyces fulvissimus*
      d^1. Aerial mycelium orange to pale pink.
         226. *Streptomyces spectabilis*
      e^1. Aerial mycelium yellowish to gray.
         86. *Streptomyces flavus*
      f^1. Aerial mycelium has olive tinge.
         122. *Streptomyces hominis*
      g^1. Aerial mycelium scant, rose-yellow.
         155. *Streptomyces microflavus*
      h^1. Aerial mycelium white to orange-colored.
         216. *Streptomyces ruber*
      i^1. Aerial mycelium gray to mouse-gray.
         34. *Streptomyces cacaoi*
   d. Growth yellowish-green to citron-yellow; aerial mycelium white to yellow to pinkish.
      56. *Streptomyces citreus*
   e. Growth colorless to cream-colored.
      a^1. Aerial mycelium scant, white.
         a^2. Good growth on milk.
            104. *Streptomyces gougerotii*
         b^2. No growth on milk.
            113. *Streptomyces griseoplanus*
      b^1. Aerial mycelium white to olive-buff.
         193. *Streptomyces praecox*
      c^1. Aerial mycelium white.
         a^2. Acid-sensitive.
            8. *Streptomyces albidus*
         b^2. Acid-resistant.
            5. *Streptomyces acidophilus*
      d^1. Aerial mycelium white to gray.
         a^2. Sporophores straight.
            249. *Streptomyces wedmorensis*
         b^2. Sporophores produce spirals.
            197. *Streptomyces pseudogriseolus*
      e^1. Aerial mycelium sandy lavender to dark gray.
         209. *Streptomyces rochei*
      f^1. Aerial mycelium rose-colored.
         215. *Streptomyces roseus*
   f. Growth black.
      99. *Streptomyces gedanensis*
   g. Growth yellow to olive-ocher.
      174. *Streptomyces olivaceus*
   h. Growth colorless to yellowish to olive-buff. Aerial mycelium water-green.
      a^1. Green and yellow pigments on malate and succinate media.
         116. *Streptomyces griseus*

b¹. No green and yellow pigments on malate and succinate media.
106. *Streptomyces griseinus*

i. Growth red or purple.
39. *Streptomyces californicus*

j. Growth colorless to black.
a¹. Aerial mycelium white to brownish-gray.
202. *Streptomyces pyridomyceticus*
b¹. Aerial mycelium on synthetic media dull gray.
157. *Streptomyces mitakaensis*

k. Growth dark brown.
a¹. Sporophores produce spirals.
a². Aerial mycelium white to gray.
a³. Growth on potato has green tinge.
118. *Streptomyces halstedii*
b³. No green tinge on potato.
219. *Streptomyces rutgersensis*
b². Aerial mycelium olive-gray.
169. *Streptomyces nodosus*
b¹. Sporophores straight.
a². Aerial mycelium gray-white.
91. *Streptomyces fumosus*
b². Aerial mycelium dark gray.
136. *Streptomyces lipmanii*

l. Growth on synthetic media rose to gray.
212. *Streptomyces roseodiastaticus*

m. Growth cream-colored to yellow or yellow-orange.
a¹. Aerial mycelium on certain media white, moist with dark, glistening patches.
124. *Streptomyces hygroscopicus*
b¹. Aerial mycelium white-yellow to brownish-yellow.
138. *Streptomyces longisporoflavus*
c¹. Aerial mycelium white.
a². Aerial mycelium present on protein media.
41. *Streptomyces candidus*
b². Aerial mycelium absent on protein media.
178. *Streptomyces omiyaensis*
d¹. Aerial mycelium powdery white, with yellow tinge.
a². Little spiral formation.
10. *Streptomyces alboflavus*
b². Abundant spiral formation.
43. *Streptomyces canus*
e¹. Aerial mycelium gray.
20. *Streptomyces antimycoticus*

n. Growth colorless to pinkish to brown.
159. *Streptomyces nagayanishii*

o. Growth orange or red.
a¹. Growth yellowish to orange; aerial mycelium seashell-pink.
a². Produces antibacterial (neomycin) and antifungal (fradicin) antibiotics.
88. *Streptomyces fradiae*
b². Produces antiviral (luridin) agent.
140. *Streptomyces luridus*
b¹. Growth rose to red; aerial mycelium white.
a². Growth yellow to red; weak proteolysis.
13. *Streptomyces albosporeus*
b². Growth pale pinkish-buff; strong proteolysis.
33. *Streptomyces brasiliensis*
c¹. Growth pale rose to red; aerial mycelium weakly developed, velvety, rose-white.
173. *Streptomyces oidiosporus*
d¹. Growth red; aerial mycelium black.
152. *Streptomyces melanocyclus*

p. Growth colorless, turning dark.
69. *Streptomyces endus*

q. Growth becoming salmon-pink; acid-sensitive.
217. *Streptomyces rubescens*

r. Growth green to dark green; aerial mycelium whitish to grayish.
245. *Streptomyces viridis*

s. Growth on blood agar brick-red.
164. *Streptomyces nitrificans*

II. Proteinaceous media are pigmented deep brown to black; melanin-positive.
  1. Growth colorless on synthetic media.
    a. Aerial mycelium thin, rose-colored.
      a¹. Spirals produced.
        210. *Streptomyces roseochromogenes*
      b¹. No spirals formed.
        54. *Streptomyces cinnamonensis*
    b. Aerial mycelium white with pale pink or pale gray tinge.
      131. *Streptomyces kitasawaensis*
    c. Aerial mycelium gray to brown to reddish.
      a¹. Growth on organic media greenish to black.
        175. *Streptomyces olivochromogenes*
      b¹. Growth dark brown.
        206. *Streptomyces resistomycificus*
      c¹. Growth cream-colored.
        163. *Streptomyces nigrifaciens*
    d. Aerial mycelium cottony, dark brown.
      66. *Streptomyces diastatochromogenes*
    e. Aerial mycelium pale yellow to gray.
      30. *Streptomyces blastmyceticus*
  2. Growth on synthetic media yellow.
    a. Aerial mycelium white.
      203. *Streptomyces rameus*
    b. Aerial mycelium white to gray.
      82. *Streptomyces flavochromogenes*
    c. Aerial mycelium white to yellow.
      46. *Streptomyces cavourensis*
    d. Aerial mycelium ash-gray.
      188. *Streptomyces pilosus*
    e. Aerial mycelium mouse-gray to green-gray.
      94. *Streptomyces galbus*
    f. Aerial mycelium hazel brown.
      139. *Streptomyces lucensis*
    g. Aerial mycelium olive-buff.
      a¹. Soluble pigment green to olive to black.
        233. *Streptomyces tenuis*
      b¹. Soluble pigment cream-colored to golden brown.
        146. *Streptomyces marginatus*
    h. Aerial mycelium white with patches of bluish-green on starch media.
      126. *Streptomyces ipomoeae*
  3. Growth white to gray.
    a. Sporophores produce spirals.
      a¹. Causes potato scab.
        223. *Streptomyces scabies*
      b¹. Does not cause potato scab.
        a². Growth on potato gray.
          119. *Streptomyces hawaiiensis*
        b². Growth on potato orange-red.
          96. *Streptomyces galtieri*
    b. Sporophores straight.
      a¹. Aerial mycelium white to gray.
        29. *Streptomyces bikiniensis*
      b¹. Aerial mycelium white, cottony.
        156. *Streptomyces mirabilis*
    c. Sporophores tend to be straight; spirals less marked.
      28. *Streptomyces beddardii*
  4. Growth cream- to brown-colored.
    a. Sporophores in clusters.
      a¹. Aerial mycelium on nutrient agar gray to yellowish-green.
        19. *Streptomyces antibioticus*
      b¹. Aerial mycelium on nutrient agar ash-gray.
        73. *Streptomyces eurythermus*
      c¹. Aerial mycelium on nutrient agar white.
        38. *Streptomyces caiusiae*
    b. Sporophores not in clusters.
      a¹. Aerial mycelium white to gray.
        109. *Streptomyces griseochromogenes*
      b¹. Aerial mycelium olive-gray.
        248. *Streptomyces viridogenes*
      c¹. Aerial mycelium olive-buff to water-green.
        107. *Streptomyces griseobrunneus*
  5. Growth red to reddish-orange.
    a. Aerial mycelium white.
      200. *Streptomyces purpurascens*

b. Aerial mycelium white to gray.
114. *Streptomyces griseoruber*
c. Aerial mycelium gray.
a¹. No soluble pigment on synthetic media.
95. *Streptomyces galilaeus*
b¹. Soluble pigment on synthetic media light carmine.
52. *Streptomyces cinereoruber*
d. Aerial mycelium scant; ability to produce such mycelium easily lost.
31. *Streptomyces bobiliae*
e. Aerial mycelium pink, with bluish-green spores.
28a. *Streptomyces bellus*

6. Growth white to cream-colored.
98. *Streptomyces garyphalus*

7. Growth buff to dark brown.
a. Aerial mycelium gray to dark olive.
105. *Streptomyces gracilis*
b. Aerial mycelium dark gray.
103. *Streptomyces globosus*
c. Aerial mycelium white.
64. *Streptomyces cylindrosporus*
d. Aerial mycelium tan to light brown.
115. *Streptomyces griseoviridis*

8. Growth on synthetic agar dark green to olive-buff.
a. Aerial mycelium white to light green to blue.
246. *Streptomyces viridochromogenes*
b. Aerial mycelium thin, white.
211. *Streptomyces roseocitreus*
c. Aerial mycelium pale gray to blue-gray.
49. *Streptomyces chartreusis*

9. Growth dark brown to black.
a. Growth on potato orange to orange-red.
a¹. No aerial mycelium on potato.
200. *Streptomyces purpurcochromogenes*
b¹. Aerial mycelium scant to none; light brownish-gray.
186. *Streptomyces phaeopurpureus*
c¹. Aerial mycelium on potato abundant, gray.
198. *Streptomyces purpureofuscus*
d¹. Aerial mycelium on potato powdery white.
132. *Streptomyces lanatus*
b. Growth on potato brown to black. Aerial mycelium on synthetic agar white to brownish.
a¹. Aerial mycelium abundant.
a². Spirals formed.
185. *Streptomyces phaeochromogenes*
b². No spirals.
153. *Streptomyces melanogenes*
b¹. Aerial mycelium on synthetic agar slight.
168. *Streptomyces noboritoensis*

10. Growth on synthetic media colorless to light orange.
a. Aerial mycelium gray to cinnamon-drab.
26. *Streptomyces aureus*
b. Aerial mycelium white to gray.
192. *Streptomyces poolensis*
c. Aerial mycelium olive-buff.
194. *Streptomyces praefecundus*

11. Growth on synthetic agar whitish-yellow to grayish-yellow.
a. Soluble pigment light yellow.
a¹. Aerial mycelium white-gray.
a². Aerial mycelium on potato gray.
231. *Streptomyces tanashiensis*
b². No aerial mycelium on potato.
78. *Streptomyces filipinensis*
b¹. Aerial mycelium olive-colored.
137. *Streptomyces loidensis*
b. Soluble pigment brown to reddish-brown.
154. *Streptomyces michiganensis*

12. Growth on synthetic agar gray to olive-gray.
44. *Streptomyces carnosus*

13. Growth on synthetic media red to purple.
a. Aerial mycelium white to gray.
71. *Streptomyces erythrochromogenes*
b. Aerial mycelium green.

6. *Streptomyces afghaniensis*

c. Aerial mycelium greenish to yellow.

4. *Streptomyces acidomyceticus*

d. Aerial mycelium chalk-white.

59. *Streptomyces collinus*

14. Growth colorless to cream-colored.

a. Aerial mycelium cottony white, lavender to vinaceous-lavender.

133. *Streptomyces lavendulae*

b. Aerial mycelium grayish-pink to lavender.

243. *Streptomyces virginiae*

c. Aerial mycelium white to cream-colored.

172. *Streptomyces odorifer*

15. Growth yellow to brown. Aerial mycelium light tan to pink.

236. *Streptomyces venezuelae*

16. Growth gray to black.

79. *Streptomyces fimbriatus*

17. Growth colorless to stone-red.

93. *Streptomyces fuscus*

18. Growth blue.

62. *Streptomyces cyaneus*

B. Sporophores in aerial mycelium form verticils.

I. Melanin-negative.

1. Growth yellowish.

a. Aerial mycelium white to pinkish.

117. *Streptomyces hachijoensis*

b. Aerial mycelium greenish-yellow.

37. *Streptomyces caespitosus*

c. Aerial mycelium gray.

128. *Streptomyces kentuckensis*

2. Growth pink to red; aerial mycelium pink.

76. *Streptomyces fervens*

3. Growth yellowish to green to brown; aerial mycelium white.

149. *Streptomyces mashuensis*

4. Growth colorless on synthetic media; aerial mycelium white to light cinnamon.

53. *Streptomyces cinnamomeus*

5. Growth colorless; aerial mycelium white.

55. *Streptomyces circulatus*

6. Growth colorless to gray; aerial mycelium white to gray.

150. *Streptomyces matensis*

II. Melanin-positive.

1. Sporophores do not produce any spirals.

a. Growth white to cream-colored.

108. *Streptomyces griseocarneus*

b. Growth colorless to yellowish.

a^1. Strong proteolytic properties.

a^2. Aerial mycelium on agar media absent or white patches.

151. *Streptomyces mediocidicus*

b^2. Aerial mycelium on agar media white, yellowish to gray.

84. *Streptomyces flavoreticuli*

b^1. Weak proteolytic action.

a^2. Aerial mycelium white to yellowish

72. *Streptomyces eurocidicus*

b^2. Aerial mycelium white to pale olive-buff.

12. *Streptomyces alboniger*

c. Growth yellowish-brown.

a^1. Aerial mycelium white.

1. *Streptomyces abikoensum*

b^1. Aerial mycelium white with yellowish tinge.

234. *Streptomyces thioluteus*

d. Growth dark gray to gray-green.

a^1. Strongly proteolytic.

238. *Streptomyces verticillatus*

b^1. Weakly proteolytic.

177. *Streptomyces olivoverticillatus*

e. Growth brown.

141. *Streptomyces luteoverticillatus*

2. Sporophores produce spirals.

a. Aerial mycelium none or limited.

232. *Streptomyces tendae*

b. Aerial mycelium yellow to ash-gray.

67. *Streptomyces echinatus*

c. Aerial mycelium white.

9. *Streptomyces albireticuli*

d. Aerial mycelium pale vinaceous.
161. *Streptomyces netropsis*

3. Sporophores straight or spiral-shaped.
207. *Streptomyces reticuli*

4. Verticils on secondary branches; growth yellowish-red to pink.
a. Spirals produced.
218. *Streptomyces rubrireticuli*
b. No spirals formed.
184. *Streptomyces pentaticus*

5. Verticils on primary and secondary branches.
a. Growth yellow to brown.
a¹. Aerial mycelium grayish-white.
130. *Streptomyces kitasatoensis*
b¹. Growth brown to olive-drab.
176. *Streptomyces olivoreticuli*
b. Growth pink.
120. *Streptomyces hiroshimensis*
c. Growth red to reddish-brown.
214. *Streptomyces roseoverticillatus*

6. Sporophores may also form tufts.
a. Aerial mycelium greenish-yellow, turning gray.
247. *Streptomyces viridoflavus*
b. Aerial mycelium white.
229. *Streptomyces spiroverticillatus*

Chapter 8

Description of Species of *Streptomyces*

Detailed descriptions of the more important, recognizable species of the genus *Streptomyces* are given in this chapter. Most of these organisms have been isolated from soils, composts, peats, and water basins; some have come from dust and food materials, from plant disease lesions, and from diseased animals and humans. Those isolated from plant disease lesions may or may not be the causative agents of such diseases; they certainly should be considered on a par with the soil-inhabiting forms. In the great majority of cases, the cultures isolated from diseased animals or from human infections as well cannot be considered as the causative agents of such diseases, since their pathogenic nature has not been established experimentally.

These descriptions vary greatly both in the details of the observations reported and in the uniformity of treatment of such observations. For many of these observations, the author had to depend on other compilers of the literature, notably Brumpt (1939), Lehmann and Neumann (1927), Dodge (1935), Krassilnikov (1949), Erikson (1935), Ettlinger *et al.* (1958), and others. Unfortunately, one cannot avoid criticizing the tendency of certain compilers to describe new species, and place others, often well recognized and previously described forms, as subspecies or as "also belonging to this species," or the even worse tendency of some classifiers to make certain minor variations the basis for establishing varieties of described organisms. In only a few cases was an attempt made to compare newly isolated cultures with previously known, although unfortunately not always available, type cultures.

Although many of the synonyms have been examined, no detailed data are presented concerning literature references. Additional information can be found in the latest edition of Bergey's Manual or in the original papers in which the descriptions have appeared.

Because of the growing interest in actinomycetes as producers of antibiotics, numerous studies of these organisms have been made during the last 5 or 6 years. Many new species and numerous new varieties have been described. Old species have been better delineated. New systems of classification have been proposed. Cooperative experiments have been carried out. All this material has now been critically examined, and much additional information has been included.

The last edition of Bergey's Manual (1957) contains descriptions of 150 *Streptomyces* species. The number has nearly doubled in the last 5 or 6 years, as indicated by the descriptions presented here.

Description of Streptomyces Species

1. *Streptomyces abikoensum* Umezawa *et al.*, 1951 (Umezawa, H., Tazaki, T., and Fukuyama, S. Japan Med. J. 4: 331–346, 1951; J. Antibiotics (Japan) 5: 469–476, 1952; Okami, Y. *ibid.* 477–480).

Morphology: Sporophores straight, short, unbranched, bearing chains of spores. No spirals. Certain strains produce verticils.

Sucrose nitrate agar: Substrate growth yellowish-brown. Aerial mycelium thin, yellowish-white. Soluble pigment yellowish-brown.

Nutrient agar: Substrate growth cream-colored to yellow. No aerial mycelium. Soluble pigment brown. Melanin-positive.

Gelatin: Growth cream-colored to brownish. Soluble pigment brown. Liquefaction crateriform.

Milk: Growth brownish. Aerial mycelium scant, white. Soluble pigment yellowish-brown. Peptonization, but no coagulation.

Potato: Growth wrinkled, cream-colored to brownish. Aerial mycelium yellowish-white. Soluble pigment reddish-brown.

Starch agar: Growth cream-colored to yellowish. Aerial mycelium white. Hydrolysis good. No soluble pigment.

Nitrate reduction: Positive.

Blood agar: Growth dark cream-yellow. Hemolysis strong.

Egg media: Growth greenish-yellow. No aerial mycelium. Soluble pigment reddish to violet.

Cellulose: Not decomposed.

Carbon utilization: Glucose, maltose, and glycerol well utilized. Arabinose, xylose, rhamnose, fructose, galactose, mannitol, sorbitol, lactose, sucrose, raffinose, and inulin not utilized.

H_2S production: Negative (other strains positive).

Tyrosinase reaction: Negative.

Antagonistic properties: Produces an antiviral agent, abikoviromycin.

Habitat: Soil in Japan.

Remarks: Resembles *S. fimicarius* and *S. purpurcochromogenes*. Gause *et al.* described a variety of this organism under the name of *A. abikoensum* var. *spiralis*. The above description was based upon strain 2-1-6.

Type culture: IMRU* 3654.

2. *Streptomyces aburaviensis* Nishimura *et al.*, 1957 (Nishimura, H., Kimura, T., Tawara, K., Sasaki, K., Nakajima, K., Shimaoka, N., Okamoto, S., Shimohira, M., and Isono, J. J. Antibiotics (Japan) **10A:** 205-212, 1957).

Morphology: Sporophores long and straight; no spirals produced. Spores oval.

Sucrose nitrate agar: Growth yellowish-brown. Aerial mycelium well developed, velvety, white. Soluble pigment dark yellowish-brown.

Glucose-asparagine agar: Growth grayish-olive, thin, flat; reverse pale olive. Aerial mycelium velvety, almost white, slightly grayish. Soluble pigment at first dull yellow, later becoming yellowish-brown.

Starch agar: Growth grayish-yellow-brown. Aerial mycelium grayish-white. Soluble pigment pale yellow-brown. Hydrolysis weak.

Calcium malate agar: Growth pale yellowish-brown. Aerial mycelium thin, white to grayish-white. Soluble pigment grayish-yellow-brown.

Nutrient agar: Growth thin, light gray. No aerial mycelium. No soluble pigment.

Milk: Growth grayish-white. Aerial mycelium white. Coagulation and peptonization.

Potato: Growth dull yellow to pale olive. Aerial mycelium white to light gray. No soluble pigment.

Gelatin: Positive liquefaction. No soluble pigment. Melanin-negative.

Nitrate reduction: Positive.

Carbon utilization: Glycerol, dextrin, starch, glucose, maltose, galactose, inulin, and fructose utilized. Mannitol, arabinose, raffinose, α-lactose, inositol, xylose, and sucrose not utilized.

3. *Streptomyces achromogenes* Okami and Umezawa, 1953 (Umezawa, H., Takeuchi, T., Okami, Y., and Tazaki, T. Japan. J. Med. Sci. Biol. **6:** 261–268, 1953).

* These designations represent the various culture collections where the type cultures are deposited. This has been elucidated in Chapter 4, p. 78–80.

Morphology: Sporophores straight, no spirals. Spores cylindrical.

Glycerol nitrate agar: Growth colorless to brownish. Aerial mycelium scant, white to dark grayish. Soluble pigment brown.

Glucose-asparagine agar: Growth yellowish brown. Aerial mycelium scant, yellowish-white. Soluble pigment none or slightly brown.

Nutrient agar: Growth wrinkled, elevated, colorless to brownish. No aerial mycelium or soluble pigment.

Potato: Growth yellowish-brown to brownish, fine, wrinkled. Aerial mycelium white, powdery. Soluble pigment absent at first, later reddish-brown.

Gelatin: Growth yellowish-brown. Soluble pigment slightly brown. Liquefaction very weak. Melanin-negative.

Milk: Surface growth poor. No soluble pigment. Coagulation and slow peptonization.

Egg media: Growth reddish-brown, wrinkled. No aerial mycelium. No soluble pigment.

Nitrate reduction: Positive.

Antagonistic properties: Produces an antiviral agent, achromoviromycin.

Remarks: This culture resembles *S. diastaticus* and *S. fimicarius*. It is characterized by the brown pigmentation on synthetic agar only. A strain of this organism, which produces the antibiotic streptozotocin, was isolated by Vavra *et al.* (1959) from a soil in Kansas; they have further cultural data concerning the original culture and the new strain.

Type culture: IMRU 3730; ATCC 12,767.

4. *Streptomyces acidomyceticus* Ogata *et al.*, 1954 (Ogata, K., Miyake, A., and Morimoto, A. Japanese Patent No. 204,403, March 5, 1954).

Morphology: Sporophores usually do not form spirals. Spores cylindrical or oval, 0.8 to 1.2 by 1.4 to 1.8 μ.

Sucrose nitrate agar: Growth at first light yellow, later dark greenish-brown. Aerial mycelium greenish to yellow-white. Soluble pigment slightly violet-colored; sometimes absent.

Glucose-asparagine agar: Growth brownish-yellow or brownish-red, and partially greenish-blue.

Calcium malate agar: Aerial mycelium greenish-white. Soluble pigment violet; sometimes absent.

Glucose nutrient agar: Growth brownish, partially dark blue. Aerial hyphae gray-white. Soluble pigment brownish-black.

Gelatin: Growth dark green. Aerial mycelium greenish-white. Soluble pigment greenish-brown. Liquefaction limited.

Potato: Growth greenish-brown. Aerial mycelium at first white, later pinkish-red. Soluble pigment dark green.

Milk: Growth cream-colored, later turning light brown. No coagulation. Soluble pigment light brown.

Starch: Slow decomposition.

Tyrosinase reaction: Negative.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Carbon utilization: Arabinose, glucose, maltose, lactose, salicin, and salts of organic acids utilized. Xylose, fructose, raffinose, inulin not attacked.

Antagonistic properties: Produces the antibiotic acidomycin.

Remarks: *S. acidomyceticus* is closely related to *S. phaeochromogenes*, the latter forming spirals in gelatin media, but not the former.

Type culture: ATCC 11,611.

5. *Streptomyces acidophilus* (Jensen, 1928) Waksman and Henrici, 1948 (Jensen, H. L. Soil Sci. **25:** 226, 1928).

Morphology: Sporophores either few or numerous, with sinistrorse spirals. Spores oval and spherical, 1.0 to 1.2 by 1.2 to 1.5 μ.

Agar media: Growth on acid media (pH 2.0 to 6.0) colorless. Aerial mycelium whitish.

Sucrose nitrate agar: No growth.

Glucose-asparagine agar: Growth raised, somewhat wrinkled, colorless in young cultures. Aerial mycelium thin, white at first, later gray or yellowish-brown.

Nutrient agar: No growth.

Starch agar: Growth at 25°C good, colorless. Aerial mycelium abundant, smooth, white. Some diastatic action.

Potato: Growth good, raised, folded. No discoloration of plug. Melanin-negative.

Gelatin: Growth after 10 days very scant, thin, semitransparent, colorless. Liquefaction slow.

Milk: No growth.

Nitrate reduction: Trace.

Sucrose: No inversion.

Antagonistic properties: Strongly positive.

Habitat: Soil.

Remarks: Grows in acid media only, with an optimum at pH 3.5 to 4.5.

6. *Streptomyces afghaniensis* Shimo *et al.* 1959 (Shimo, M., Shiga, T., Tomosugi, T., and Kamoi, I. J. Antibiotics (Japan) **12A:** 1, 1959).

Morphology: Sporophores form spirals.

Sucrose nitrate agar: Growth olive-colored, with reddish-brown reverse. Aerial mycelium pale green to light greenish-gray. Soluble pigment brown to reddish-brown.

Glucose-asparagine agar: Growth olive-colored, with reddish-brown reverse. Aerial mycelium pale green to light greenish-gray. Soluble pigment brown to reddish-brown.

Calcium malate agar: Growth olive-colored. Aerial mycelium pale yellow-orange to pale orange. Soluble pigment yellowish-brown to reddish-brown.

Nutrient agar: Growth colorless to olive to buff. Aerial mycelium grayish-white. Soluble pigment light brown.

Gelatin: Growth colorless. Aerial mycelium white. Soluble pigment brown. Medium liquefaction.

Milk: Growth yellowish-brown. No aerial mycelium. Soluble pigment brown to dark brown.

Potato: Growth wrinkled, colorless. Aerial mycelium olive to yellowish-brown. Soluble pigment yellowish-brown.

Cellulose: Positive growth.

Nitrate reduction: Negative.

Tyrosinase: Doubtful.

Carbon source: Utilizes rhamnose, raffinose, and other carbohydrates; does not utilize sodium citrate and sodium acetate; doubtful growth on dulcitol and sorbitol.

Antagonistic properties: Produces an antibiotic, taitomycin, active upon gram-positive bacteria.

Habitat: Soil in Afghanistan.

Remarks: Resembles *S. collinus* and *S. erythrochromogenes.*

7. *Streptomyces albidoflavus* (Rossi-Doria, 1891, *emend.* Gasperini, 1894) Waksman and Henrici, 1948 (Rossi-Doria, T. Ann. ist. ig. sper. Roma, n. s. **1:** 399–438, 1894).

Synonym: *Actinomyces albido-flavus* Duché, 1934, *emend.* Krassilnikov, 1949.

Morphology: Sporophores short, spiral-forming, sinistrorse. Spores spherical.

Glucose-asparagine agar: Growth brown. Aerial mycelium white, later becoming whitish-yellow. Soluble pigment yellowish.

Glucose-peptone agar: Growth cream-colored, covered with fine white aerial mycelium; yellow soluble pigment.

Tyrosine agar: Growth fine with orange-yellow on reverse side; medium becomes yellowish to yellowish-rose.

Gelatin: Punctiform colonies with white aerial mycelium on surface. No soluble pigment. Rapid liquefaction.

Milk: Growth rapid, becoming covered with whitish aerial mycelium; never fully covering the surface; no coagulation; peptonization begins slowly and is completed in 13 days; liquid colored yellowish-orange.

Starch media: Growth cream-colored, covered with yellow aerial mycelium. After 20 days, growth becomes much folded; greenish on reverse side; soluble pigment slightly amber. Hydrolysis.

Cellulose: Some growth.

Coagulated serum: Cream-colored growth on surface. Aerial mycelium white. Liquefaction rapid.

Production of H_2S: Negative.

Antagonistic properties: Produces streptothricin.

Habitat: Soil.

Remarks: According to Flaig and Kutzner (1960), this culture obtained from CBS is *S. coelicolor* Müller. Ettlinger *et al.* (1958) considered that Duché's strain of this organism belongs to *S. griseus*.

8. *Streptomyces albidus* (Duché, 1934) Waksman (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Morphology: Sporophores form long, open spirals. Spores spherical to oval.

Glucose nitrate agar: Growth colorless; some drops of colorless guttation. Aerial mycelium white. Soluble pigment yellowish.

Peptone agar: Growth colorless. Aerial mycelium white; reverse slightly greenish. Soluble pigment brownish.

Potato: Growth flat, colorless. Aerial mycelium white. No soluble pigment.

Gelatin: Growth cream-colored. Rapid liquefaction. No soluble pigment. Melanin-negative.

Milk: Growth cream-colored. Coagulation weak; peptonization rapid. Odor cheesy.

Starch: Hydrolysis good.

Cellulose: Growth good.

Fats and waxes: Growth good, according to Krassilnikov (1949).

Nitrate: Slow reduction to nitrate.

Odor: Strong, earthy.

Antagonistic properties: According to Krassilnikov (1949), it possesses strong antagonistic activities.

Remarks: Closely related to *S. albus* (Krassilnikov, 1949); differs by more delicate growth, by a reverse that is often yellowish-brown. Also related to *S. microflavus*, but differs from the form described by Krainsky in that its growth is never rose-yellow and that it grows abundantly on potato. Gause *et al.* (1957) described a variety of this organism under the name *A. albidus* var. *invertens*. Ettlinger *et al.* (1958) considered it as a strain of *S. griseus*.

9. *Streptomyces albireticuli* Nakazawa, 1955 (Nakazawa, K. J. Agr. Chem. Soc. Japan **29:** 644–647; 647–649, 1955).

Morphology: Produces spirals in the secondary verticils of the aerial mycelium. The spores are cylindrical 0.6 to 0.8 by 1.4 to 1.8 μ.

Sucrose nitrate agar: Growth thin, colorless; reverse pale ochraceous salmon. Aerial mycelium white.

Glucose-asparagine agar: Growth colorless; later becoming yellow. Aerial mycelium white, cottony, later becoming cream-colored.

Nutrient agar: Growth thin, mouse-gray. No aerial mycelium. Soluble pigment ochraceous tawny.

Potato plug: Growth gray. Aerial mycelium white. Color of plug brown.

Gelatin: Liquefaction slow. Soluble pigment black.

Milk: Growth cream-colored. Peptonization slow. Soluble pigment brown after 24 days.

Starch: Actively diastatic.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Cellulose: No growth.

Antagonistic properties: Produces eurocidin, an antifungal antibiotic.

10. *Streptomyces alboflavus* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 99–134, 1916; **8:** 90, 1919).

Morphology: Sporophores straight, branching, with very little tendency to produce spirals. Spores oval-shaped.

Sucrose nitrate agar: Growth glossy, spreading, colorless, becoming yellowish. Aerial mycelium powdery, white, with yellowish tinge. No soluble pigment.

Glycerol malate agar: Growth light pinkish-cinnamon. Aerial mycelium late, white.

Glucose-asparagine agar: Growth restricted, much folded, cream-colored with sulfur-yellow surface. No aerial mycelium. No soluble pigment.

Nutrient agar: Growth restricted, cream-colored. No aerial mycelium. No soluble pigment.

Potato: Growth wrinkled, moist, cream-colored.

Gelatin: Surface growth abundant, colorless. Aerial mycelium white or absent. No soluble pigment. Slow liquefaction.

Milk: Surface ring pinkish. No coagulation; limited peptonization.

Starch media: Growth thin, spreading, yellowish. No aerial mycelium. Good hydrolysis of starch.

Cellulose: Scant growth.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Positive.

Remarks: Various cultures related to this organism have been described under a variety of different names. It is sufficient to mention *A. cremeus*, *A. griseoloalbus*, *A. flavidovirens*, and a variety of the latter, *fuscus*, described by Gause *et al.* (1957). Krassilnikov (1949) considered it as a variety of *A. flavus*.

Type culture: IMRU 3008.

11. *Streptomyces albogriseolus* Benedict *et al.*, 1954 (Benedict, R. G., Shotwell, O. L., Pridham, T. G., Lindenfelser, L. A., and Haynes, W. C. Antibiotics & Chemotherapy **4:** 653–656, 1954).

Morphology: Sporophores monopodially branched, producing short, compact spirals, averaging 4 to 6 turns. Spores spherical or oval, covered with numerous long, fine hairs (Pl. II n).

Sucrose nitrate agar: Aerial mycelium white, becoming ash-gray, often with white spots.

Starch agar: Aerial mycelium white to dark gray. Hydrolysis.

Nutrient agar: Aerial mycelium white to ash-gray. Melanin-negative.

Potato: Growth cretaceous to dirty grayish-white to faint pink.

Carrot: Vegetative growth white to dirty cream; no aerial mycelium. Slant not darkened.

Gelatin: Dirty white sediment. Positive liquefaction. Not pigmented.

Milk: Orange-colored ring; partially peptonized at 14 days.

Nitrate: Reduction to nitrite.

Production of H_2S: Negative.

Temperature: Good growth at 25–41°C. No growth at 50°C.

Antagonistic properties: Produces a "neomycin complex."

Habitat: Soil.

Type culture: IMRU 3698.

12. *Streptomyces alboniger* Hesseltine *et al.*, 1954 (Hesseltine, C. W., Porter, J. N., Deduck, N., Hauck, M., Bohonos, N., and Williams, J. H. Mycologia **46:** 16–23, 1954).

Morphology: Sporophores irregularly branched, erect to flexuous; no spirals. Verticils produced. Spores catenulate, oval, 0.8 by 1.25 μ.

Sucrose nitrate agar: Growth poor, white. Aerial mycelium white to pale olive-buff. No soluble pigment.

Glucose-asparagine agar: Growth blackish-gray. Aerial mycelium white. Soluble pigment blackish-gray.

Nutrient agar: Growth moist, smooth, colorless to yellowish, to dark brown or black. No aerial mycelium. No soluble pigment.

Starch agar: Growth good. Aerial mycelium white to pale olive-buff. Soluble pigment black. Good hydrolysis.

Potato: Growth moist, yellow. Aerial

mycelium white. Soluble pigment dark, greenish-black.

Gelatin: Growth fair. Aerial mycelium white. Soluble pigment light yellow. Liquefaction medium.

Milk: Surface white ring, with yellow-green to light yellow-brown below surface. Aerial mycelium white. Peptonization slow.

Cellulose: No growth.

Production of H_2S: Negative.

Antagonistic properties: Produces puromycin, an antibiotic active upon certain gram-positive bacteria and protozoa.

Habitat: Forest soil.

Remarks: Culture is characterized by the formation of an olivaceous black soluble pigment in some media, such as asparagine-glucose agar, but no such pigment is produced on certain organic media.

Type culture: ATCC 12,461.

13. *Streptomyces albosporeus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 687, 1914; Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 99, 1916; **8:** 90, 1919).

Morphology: Sporophores straight, branching, with occasional spirals. Spores spherical or oval, 0.8 to 1.2 by 1.0 to 1.8μ.

Sucrose nitrate agar: Growth spreading, colorless, with pink center, becoming brownish, vinaceous. Aerial mycelium white, covering the whole surface; often none. No soluble pigment.

Glycerol malate agar: Growth rose to orange-red. Aerial mycelium white, later changing to yellow. No soluble pigment.

Glucose-asparagine agar: Growth wrinkled, spreading, red, with colorless margin. Aerial mycelium appears late, white.

Nutrient agar: Small, cream-colored colonies. No aerial mycelium. No soluble pigment.

Starch agar: Growth thin, spreading, transparent, with red tinge. No aerial mycelium. Ready hydrolysis.

Potato: Growth red to brownish-gray. No aerial mycelium, or white. Melanin-negative.

Gelatin: Growth yellow, changing to red, with hyaline margin. Usually no aerial mycelium; when produced, sometimes gray. Medium liquefaction.

Milk: Scant, pink ring. No coagulation; no peptonization.

Cellulose: No growth or scant.

Nitrate reduction: Fair.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Positive.

Habitat: Soil.

Remarks: Above description is based partly upon the isolates of Waksman and Curtis, since Krainsky's culture was not available. Krassilnikov (1949) considered it as a variety of *A. ruber*. According to Ettlinger *et al.* (1958) this organism should be considered as a strain of *S. griseus*, a hardly justifiable assumption.

Type culture: IMRU 3003.

14. *Streptomyces alboviridis* (Duché, 1934) Waksman (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, p. 317, 1934).

Morphology: According to Krassilnikov (1949), the sporophores produce spirals with 3 to 4 turns. Spores spherical.

Glucose nitrate agar: Growth cream-colored becoming olive-green. Aerial mycelium white, becoming yellowish-green. Soluble pigment brownish.

Glucose-asparagine agar: Growth at first white, becoming olive-colored to almost dark. Aerial mycelium white to green. Soluble pigment yellowish.

Starch agar: Growth cream-colored; reverse brownish-green. Aerial mycelium white, becoming green.

Gelatin: Growth white, becoming green. Soluble pigment greenish-brown. Liquefaction rapid (slow, according to Krassilnikov, 1949).

Potato: Growth white, becoming brownish to rust-colored. Plug colored black.

Tyrosine agar: Growth white with a brownish reverse. Soluble pigment brownish.

Milk: Coagulation and peptonization.

Coagulated serum: Growth cream-colored. No aerial mycelium. No soluble pigment. Rapid liquefaction of serum.

Remarks: This organism is considered as a transitional form between *S. albus* and *S. viridis*. Krassilnikov (1949) considered it as a variety of *A. viridochromogenes*.

15. *Streptomyces albus* (Rossi-Doria, 1891; *emend.* Gasperini, 1892) Waksman and Henrici, 1948 (Rossi-Doria, T. Ann. ist. ig. sper. Roma, n. s. **1:** 399–438, 1894).

Synonym: Numerous synonyms of this species are found in the literature. They belong mostly to the species-group "*S. albus*." Many of them are listed in Chapter 6, under the corresponding group.

Morphology: Sporophores produce long spirals. Spores spherical to oval. Some strains produce, according to Okami, straight sporophores, depending on the composition of the medium.

Agar media: Growth colorless; may become yellowish to brown with age. No soluble pigment formed, although some strains may excrete a brownish substance in certain media and under certain conditions. Aerial mycelium abundant, white; the shade of color varies with composition of medium from snow-white to somewhat yellowish.

Sucrose nitrate agar: Substrate growth smooth, colorless. Aerial mycelium cottony to powdery; white to snow-white.

Glucose-asparagine agar: Aerial mycelium gray, becoming brownish.

Nutrient agar: Generally no aerial mycelium; chalky white deposit on old colonies.

Potato: Growth lichenoid, cream-colored. Aerial mycelium white.

Gelatin: Colonies gray. No soluble pigment. Strong liquefaction.

Milk: Surface ring cream-colored. Aerial mycelium white. Peptonization rapid.

Starch agar: Aerial mycelium white. Rapid hydrolysis of starch in some cultures; others show little or no hydrolysis.

Nitrate: Reduction to nitrite positive.

Production of H_2S: Negative.

Odor: Characteristic, moldy.

Antagonistic properties: Certain strains are active upon gram-positive bacteria. Some produce actinomycetin, others form thiolutin or endomycin.

Habitat: Occurs in dust and soil.

Remarks: The general occurrence of this species, the ease of its superficial identification, and the fact that it has been adopted as the type species for the genus *Streptomyces*, justify a more complete characterization, as given in Chapter 6. Numerous strains of this species, varying in their cultural and other properties have been reported. Numerous descriptions of closely related organisms also are found in the literature (Duché). Krassilnikov lists 18 strains and substrains (*A. albus vulgaris*, *A. albus chlamydosporus*, etc.). *A. longisporus* Krassilnikov (1949) and some of the substrains, like *A. longisporus griseus*, belong to this group. Solovieva and Rudaya (Antibiotiki, **4(6):** 5–10, 1959) list a variety *fungatus* capable of producing an antifungal agent, albofungin.

Type culture: IMRU 3005.

16. *Streptomyces althioticus* Yamaguchi *et al.*, 1957 (Yamaguchi, H., Nakayama, Y., Takeda, K., Tawara, K., Maeda, K., Takeuchi, T., and Umezawa, H. J. Antibiotics (Japan) **10A:** 195–200, 1957).

Morphology: Curved chains or spirals of oval spores on ends of aerial sporophores. Frequently, tips of aerial mycelium divided into tufts of spore chains.

Sucrose nitrate agar: Growth colorless to white, later light brown to purplish. Aerial mycelium powdery white, later gray.

Glucose-asparagine agar: Growth colorless to white, later light brown with or without dull light reddish tinge. Surface glossy. Aerial mycelium scant, white. Light brown to dull light reddish-brown soluble pigment.

Starch agar: Aerial mycelium white to gray. No soluble pigment. No hydrolysis in 7 days.

Glucose-asparagine agar: Growth colorless to white, later light brown with or without dull light reddish tinge. Surface glossy. Aerial mycelium scant, white. Light brown to dull light reddish-brown soluble pigment.

Glucose nutrient agar: Growth light yellowish-brown. Surface glossy. Aerial mycelium white to gray. Soluble pigment light yellowish-brown.

Potato: Growth abundant, light yellowish-brown. Aerial mycelium white to gray.

Gelatin: Scant growth. No liquefaction. Brown soluble pigment.

Milk: Light yellowish-brown surface ring, with scant white aerial mycelium. Yellowish-orange soluble pigment occasionally. Peptonization positive.

Egg medium (37°C): Growth yellow with gray tinge. Aerial mycelium white, later light purplish occasionally.

Cellulose: Scant growth with purplish-gray aerial mycelium and light purplish pigment.

Carbon utilization: Abundant growth with rhamnose, fructose, galactose, mannitol, and glucose; weak growth with xylose, arabinose, maltose, sorbitol, and inositol; none or very scant with dulcitol, raffinose, and inulin.

Antagonistic properties: Produces antibiotic althiomycin.

Remarks: Closely related to *S. achromogenes* and *S. rimosus*. Spiral formation of culture, no nitrite formation, and purplish tone of growth and aerial mycelium differentiate it from *S. achromogenes*. Purplish tinge of aerial mycelium and growth, soluble pigment, no nitrite formation, and no cracked surface of the growth differentiate it from *S. rimosus*.

17. *Streptomyces ambofaciens* Pinnert-Sindico, 1954 (Pinnert-Sindico, S. Ann. inst. Pasteur **87:** 703–707, 1954).

Morphology: Sporophores form spirals. Spores oval or spherical.

Sucrose nitrate agar: Substrate growth yellow to gray. Aerial mycelium white to gray. Soluble pigment weak brownish-yellow.

Glucose-asparagine agar: Growth yellow, covered with white aerial mycelium. Soluble pigment weak yellow-brown.

Calcium malate agar: Growth similar to that on sucrose nitrate agar. No soluble pigment.

Potato: Growth clear brown. Aerial mycelium powdery gray. Soluble pigment weakly brown to brownish-red.

Gelatin: Surface growth yellow; flakes in liquefied portion. Medium liquefaction. Weak brown-orange pigment in liquefied zone. Melanin-negative.

Milk: No coagulation, partial peptonization in 1 month. Peptonized zone orange-brown to red.

Nitrate: Weak reduction to nitrite in synthetic media; none at all in organic media.

Production of H_2S: Negative.

Carbon utilization: Glycerol, arabinose, glucose, galactose, levulose, mannose, lactose, rhamnose, starch, and mannitol well utilized. Raffinose, erythritol, dulcitol, and sorbitol not utilized.

Antagonistic properties: Produces two antibiotics, congocidin and spiramycin.

Remarks: Ettlinger *et al.* (1958) included this organism with *S. aureofaciens*.

18. *Streptomyces annulatus* (Beijerinck, 1912; *emend.* Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk, SSSR, Moskau, 1941).

Not *A. annulatus* Wollenweber, 1920.

Morphology: Sporophores produce spirals, with 3 to 7 turns (sinistrorse). Spores spherical, 0.7 μ.

Sucrose nitrate agar: Growth colorless, flat, penetrating deep into agar. Aerial mycelium white, velvety, growing in the form of concentric rings.

Nutrient agar: Colorless growth. Aerial mycelium white, concentric rings less marked. Melanin-negative.

Gelatin: Slow liquefaction.

Milk: Positive coagulation and slow peptonization.

Starch: Hydrolysis.

Cellulose: Growth good.

Invertase: Positive.

Production of H_2S: Negative.

Odor: Strong, earthy.

Antagonistic properties: Highly antagonistic to mycobacteria and gram-positive bacteria; some strains are active against fungi.

Habitat: Soil.

Remarks: Krassilnikov (1949) considers this organism as a variety of *S. albus*.

Type culture: IMRU 3307.

19. *Streptomyces antibioticus* (Waksman and Woodruff, 1941) Waksman and Henrici, 1948 (Waksman, S. A. and Woodruff, H. B. J. Bacteriol. **42:** 232, 246, 1941; see also Waksman, S. A. and Gregory, F. J. Antibiotics & Chemotherapy **4:** 1050–1056, 1954).

Morphology: Sporophores straight, long, arranged in clusters or broom-shaped bodies; usually not wavy and no spirals; some strains may produce a few spirals. Spores nearly spherical to somewhat elliptical, smooth (Pl. II 1). Capacity to produce aerial mycelium may be lost upon continued cultivation on artificial media (Pl. V Fb).

Sucrose nitrate agar: Growth cream-colored to yellowish, tending to darken in reverse. Aerial mycelium light to mouse-gray, with white patches. Soluble pigment faint yellowish to yellow to dark.

Glucose-asparagine agar: Growth cream-colored, with yellowish to orange to dark reverse. Aerial mycelium light to ash-gray. Soluble pigment absent or yellow to brownish.

Calcium malate agar: Growth colorless to yellowish. Aerial mycelium white to white-gray.

Nutrient agar: Growth brownish, thin. Aerial mycelium yellowish-gray to yellowish-green. Soluble pigment brown to dark. Melanin-positive.

Potato: Growth thin to heavy, lichenoid; brownish to orange in color, sometimes olive-green. Aerial mycelium absent or thin to patchy, white or gray. Soluble pigment brownish to dark; absent in many cultures.

Gelatin: Growth yellowish to brown to dark brown. Aerial mycelium as patches of white to gray. Soluble pigment black. Liquefaction at first very slow, later becoming more rapid.

Milk: Thick surface ring, brownish. Aerial mycelium mouse-gray with greenish tinge. No coagulation, but gradual peptonization. Soluble pigment brownish to black.

Production of H_2S: Positive.

Tyrosinase: Negative.

Antagonistic properties: Marked antagonistic effect on bacteria and fungi. Produces actinomycin A, the first crystalline antibiotic ever isolated from an actinomycete culture.

Source: Isolated from soil on *Escherichia coli*-washed plate, using living cells of *E. coli* as the only source of available nutrients. Later also isolated from a variety of different soils.

Remarks: Ettlinger *et al.* (1958) included in this group *S. bikiniensis*, *S. cinereoruber*, *S. eurythermus*, and *S. ipomoeae*. Krassilnikov (1949) included this species with *A. parvus*.

Type culture: IMRU 3435.

20. *Streptomyces antimycoticus* Waksman

(Leben, C., Stessel, G. J., Keitt, G. W. Mycologia **44:** 159–169, 1952).

Morphology: Sporophores with spirals situated typically in dense groups. Spirals tend to be open, becoming closed and compact prior to the formation of spores. Spores oval, 0.6 to 1.3 by 0.7 to 2.0 μ.

Sucrose nitrate agar: Substrate growth at first white, later gray. Aerial mycelium abundant, light neutral gray. No soluble pigment.

Glycerol malate agar: Aerial mycelium abundant, light neutral gray. Soluble pigment faint green.

Nutrient peptone agar: Growth shiny, cream-colored. Aerial mycelium moderate, pebbly, white. No soluble pigment. Melanin-negative.

Potato-glucose agar: Aerial mycelium abundant, neutral gray. Soluble pigment faint, brown.

Yeast extract agar: Aerial mycelium abundant, neutral gray. No soluble pigment.

Starch agar: Aerial mycelium abundant, white to neutral gray. No soluble pigment. Diastatic action weak to moderate.

Potato: Growth finely wrinkled, cream-colored. Aerial mycelium sparse. Plug darkened slightly.

Gelatin: Growth translucent, cream-colored. Aerial mycelium sparse, white. Liquefaction slight at 15 days, moderate at 30 days. No soluble pigment.

Milk: Ring cream-colored. Coagulation; peptonization in 15 to 30 days. Yellowish-orange pigmentation.

Nitrate reduction: Slight.

Antagonistic properties: Produces an antifungal agent, helixin.

21. *Streptomyces arenae* Grundy, 1954 (Grundy, W. E. Brit. Pat. 719,230, Dec. 1, 1954*).

Morphology: Monopodial branching of mycelium. Sporophores terminate in tight spirals. Spores spherical to oval.

Sucrose nitrate agar: Growth wrinkled, yellow, turning dark orange-brown with age. Aerial mycelium grayish-white. Soluble pigment light yellow-brown.

Calcium malate agar: Growth cream-colored, turning bright reddish-brown with age. Aerial mycelium fluffy, cream-colored turning gray with pink tinge. Soluble pigment light pink. Complete dissolution of the calcium malate.

Glucose-asparagine agar: Growth sparse, golden brown; a few tufts of white aerial mycelium. Soluble pigment yellow.

Nutrient agar: Growth moderate, golden brown. Aerial mycelium gray-white, spores turning darker gray. Soluble pigment light brown.

Potato: Growth abundant, golden brown, turning dark brown. Aerial mycelium fluffy, becoming on sporulation dark gray. Potato dark gray, turning black.

Gelatin: Heavy gray pellicle on surface. Liquefaction slow. Soluble pigment deep red-brown diffusing through the liquefied zone. Medium liquefaction.

Milk: Heavy pellicle. Milk digested in 25 to 28 days with formation of curd just before complete digestion. Soluble pigment dark brown, turning black in 30 days.

Starch: Hydrolysis slow.

Nitrate: No reduction.

Carbon utilization: Good growth with xylose, glucose, mannose, galactose, lactose, maltose, sucrose, starch, mannitol, glycerol sodium acetate, sodium citrate, and potassium sodium tartrate. Sorbitol and calcium lactate not utilized.

Antagonistic properties: Produces an antibiotic active upon *Mycobacterium tuberculosis*.

Habitat: Illinois soil.

22. *Streptomyces argenteolus* Perlman, 1957 (Perlman, D. U. S. Patent 2,709,705, October 7, 1958).

Morphology: Aerial mycelium hyaline, generally branched, not forming loops or

* Supplemented by personal communication.

spirals; individual filaments are rarely septate. Sporophores straight, flexuous, or fascicled (in tufts). Spores are oval to oblong, 1.0 to 1.2 μ. The spore color is light gull-gray.

Sucrose nitrate agar: No growth.

Nutrient agar: Growth colorless, abundant, spreading. Aerial mycelium white. No soluble pigment.

Oatmeal agar: Growth good. Aerial mycelium limited, no sporulation. Soluble pigment slight maize-yellow.

Casein digest-meat extract agar: Growth abundant, dark olive-buff. Aerial mycelium well developed, pale smoke-gray. No soluble pigment.

Gelatin: Rapid liquefaction. Melanin-negative.

Milk: Positive coagulation and peptonization.

Potato: Growth good, creamy-buff, cerebriform. Aerial mycelium white; no sporulation. No soluble pigment.

Starch: Hydrolyzed.

Nitrate reduction: Positive.

Carbon utilization: Mannitol, *d*-xylose, *l*-arabinose, *l*-rhamnose, *d*-fructose, trehalose, and lactose utilized. No growth or very scant growth with inositol, sorbitol, melibiose, sucrose, and dextrin.

Habitat: Soil.

Biochemical activities: Certain strains of this organism convert progesterone to 16 α-hydroxyprogesterone.

23. *Streptomyces armillatus* Mancy-Courtillet and Pinnert-Sindico, 1954 (Mancy-Courtillet, D. and Pinnert-Sindico, S. Ann. inst. Pasteur **87:** 580–584, 1954).

Morphology: Aerial mycelium produces spirals.

Glucose- or glycerol-asparagine agar: Growth yellow-gray. Aerial mycelium poorly developed, white.

Sucrose nitrate agar: Growth very poor, colorless. No aerial mycelium.

Glucose nitrate agar: Growth poor. No soluble pigment. No reduction of nitrate.

Glucose-peptone agar: Growth very good, yellow-gray. Aerial mycelium poorly developed, white. Soluble pigment weak rose, becoming brownish. Melanin-negative.

Potato: Growth good, yellow-gray. Aerial mycelium poorly developed. No soluble pigment.

Tyrosine medium: Growth flat, yellow-gray, becoming beige. Aerial mycelium white. No soluble pigment.

Gelatin: Growth in form of pellicle. Aerial mycelium white. Soluble pigment rose-brown. Rapid liquefaction.

Milk: Growth in form of surface ring, gray to yellow. Peptonized portion colored yellow. Coagulation and rapid peptonization.

Starch: No hydrolysis.

Antagonistic properties: Produces oxytetracycline.

Remarks: This organism can be classified with the *S. bobiliae-S. erythreus* group, although its growth is yellow rather than red. It grows poorly upon synthetic media, upon which it forms no aerial mycelium. It does not reduce nitrate to nitrite. It does not produce a purple pigment upon egg media. It does not change the reaction of milk to alkaline. It does not hydrolyze starch. It differs from *S. rimosus* and *S. griseoflavus*, which produce yellow to brown pigments; *S. armillatus* under the same conditions does not form any pigment.

24. *Streptomyces aurantiacus* (Rossi-Doria, 1891 *emend.* Gasperini, 1892; *emend.* Krassilnikov) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 36, 1941).

Morphology: Produces an abundance of chlamydospores. Sporophores form spirals with 3 to 5 turns. Spores spherical to oval, 0.7 to 0.9 by 0.6 to 0.8 μ.

Agar media: Growth lichenoid, dry, compact; colored bright orange or golden. Pigment insoluble in medium, but soluble in

organic solvents. Aerial mycelium poorly developed. Melanin-negative.

Potato: Soluble pigment brown.

Gelatin: Growth yellow to orange-yellow to deep orange. Liquefaction none or slow. No aerial mycelium.

Milk: Surface growth orange. No coagulation; unchanged or weak peptonization.

Starch: Slow hydrolysis.

Cellulose: No growth.

Nitrate: No reduction.

Invertase: None.

Fats: Hydrolysis and utilization rapid.

Paraffin: Growth good, with spiral-forming sporophores and spherical spores.

Pigment: Red-orange, extracted with 96 per cent alcohol. The orange pigment was dissolved in petroleum ether, the red pigment being insoluble (Kriss).

Antagonistic properties: Strongly antagonistic to gram-positive bacteria.

Habitat: Soil, dust.

Remarks: Some strains deposit ferric oxide on the surface of the hyphae.

25. *Streptomyces aureofaciens* Duggar, 1949 (Duggar, B. M. Ann. N. Y. Acad. Sci. **51:** 177, 1948; U. S. Patent 2,482,055, Sept. 14, 1949).

Morphology: Sporophores monopodially branched, flexuous, producing open spirals. Spores spherical to oval, smooth (Pl. II m).

Sucrose nitrate agar: Substrate growth only. Occasionally faint brownish pigment produced.

Glucose-asparagine-meat extract agar: Growth hyaline, changing to orange-yellow or purplish-brown. Aerial mycelium, if present, white, changing to ash-gray or dark gray with tawny reverse. Faint yellowish soluble pigment occasionally discernible.

Nutrient agar: Growth good, translucent to brownish. No aerial mycelium. No soluble pigment. Melanin-negative.

Potato: Growth lichenoid, light orange-yellow to brown-red to purplish. No aerial mycelium. Color of plug unchanged.

Gelatin: Cream-colored surface ring. Liquefaction none to limited. No soluble pigment.

Milk: Growth limited, yellow-brown surface. Coagulation and peptonization variable (often none, occasionally present).

Production of H_2S: Mostly negative.

Antagonistic properties: Produces chlortetracycline, an amphoteric compound containing both nitrogen and non-ionic chlorine, active against various bacteria, rickettsiae, and the larger viruses. The organism also produces, especially in a chlorine-poor medium, tetracycline. The presence of phosphorus in the medium influences not only growth but also antibiotic production (Prokofieva-Belgovskaya and Popova, 1959).

Habitat: Soil.

Remarks: The numerous natural and induced variants of *S. aureofaciens* display wide variations in color of substrate growth, ranging from pale yellow to reddish-brown, and even occasionally greenish, depending upon the composition of the nutrient substrates and environmental conditions (Duggar *et al.*, 1954). Color of aerial mycelium is influenced by sporulation. Ettlinger *et al.* (1958) included *S. ambofaciens* in this group.

Type culture: IMRU 3550; ATCC 10,762.

26. *Streptomyces aureus* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 24, 1916; **8:** 97, 1919).

Morphology: Aerial mycelium forms sporophores with numerous closed spirals; some strains produce flexible sporophores with open spirals. Spores spherical to oval, 0.6 to 1.0 by 0.8 to 1.4 μ (Fig. 32).

Sucrose nitrate agar: Growth thin, spreading, colorless, becoming dark brown. Aerial mycelium thin, powdery, mouse-gray, becoming cinnamon-drab. No soluble pigment.

Malate-glycerol agar: Growth cream-colored, with surface almost black. Aerial mycelium light brown. No soluble pigment.

Glucose-asparagine agar: Growth light orange; raised center, hyaline margin. Aerial mycelium light drab.

Nutrient agar: Growth restricted, gray. No aerial mycelium. Soluble pigment deep brown.

Starch agar: Growth thin, transparent, spreading. Aerial mycelium buff-colored. Good hydrolysis.

Potato: Growth abundant, wrinkled, brown, becoming black. Aerial mycelium white to ash-gray. Soluble pigment black.

Gelatin: Surface growth fair, cream-colored, becoming brown. Aerial mycelium

FIGURE 32. Sporophores of *S. aureus*, × 30,000 (Courtesy of E. Baldacci, University of Milan, Italy).

absent or white. Brown soluble pigment. Liquefaction rapid, later slowing down.

Milk: Black ring. Limited coagulation and peptonization.

Nitrate: Reduction to nitrite with certain carbon sources.

Invertase: None to positive.

Temperature: Optimum 25°C.

Antagonistic properties: Produces polyenes, substances active against various fungi. Some strains produce luteomycin.

Habitat: Soil.

Remarks: Cultures under this name were described by DuBois-Severin in 1895, by Lachner-Sandoval in 1899, and by Sartory in 1923. Yamaguchi and Saburi (1955) reported that the *S. aureus* culture obtained from collections produces straight aerial hyphae and no spirals when grown on various synthetic media. Okami and Suzuki (1958) isolated two strains that produced spirals. Ettlinger *et al.* (1958) considered this organism as a strain of *S. griseus*. Krassilnikov (1949) considered it as a variety of *A. flavus*.

Type culture: IMRU 3309.

27. *Streptomyces autotrophicus* Takamiya and Tubaki, 1956 (Takamiya, A., and Tubaki, K. Arch. Mikrobiol. **25:** 58–64, 1956).

Morphology: Sporophores alternately or irregularly branched, breaking up into spores; no spiral formation. Spores colorless with smooth surface; varying in shape from ellipsoid to long ovoid or cylindrical; usually 2.5 to 4.3 by 0.5 to 0.8 μ, sometimes smaller, 1.5 by 0.3 μ.

Nitrate, carbohydrate-free, agar: Aerial mycelium powdery and snow-white in appearance, consisting of a tough mycelial felt; thicker at central area than at periphery. Reverse side of growth wrinkled and pale yellowish. No soluble pigment.

Calcium malate agar: Growth much folded, and raised in central area, cream-yellow at earlier stages of development; reverse side pale brownish. Aerial mycelium white.

Nutrient agar: Growth much folded and raised; reverse side relatively smooth and pale brownish. Aerial mycelium snow-white. In old cultures, a faint brown tint in agar layer immediately beneath growing colony.

Malt agar: Growth irregularly wrinkled and folded; reverse side wrinkled and pale yellowish. Production of spores rather poor.

Starch agar: Growth scanty; no hydrolysis.

Potato: Growth colorless, much folded, with thick central area and thin periphery, pale brownish.

Gelatin: No liquefaction.

Milk: Thin white pellicle formed on surface. Reverse side yellowish. No coagulation. No soluble pigment.

Cellulose: Not decomposed.

Nitrate reduction: None.

Habitat: Originally found on the surface of phosphate buffer solution left unused in a laboratory in Tokyo. Conceivably, it was derived from atmospheric dust.

Remarks: Hirsch (1960) considers this organism as a *Nocardia* (*N. autotrophica*), capable of utilizing petroleum.

28. *Streptomyces beddardii* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council (Brit.) Spec. Rept. Ser. **203:** 13–14, 1935).

Morphology: Sporophores long, slender, forming many wavy or closely coiled spirals, particularly on glucose agar; spirals less marked or lacking on poorer nutritive media like synthetic glycerol agar or water agar. Aerial hyphae straighter and more branched with shorter sporophores on starch agar. Spores oval.

Glucose-asparagine agar: Growth wrinkled, membranous. Aerial mycelium scant, white.

Nutrient agar: Growth colorless, coherent, wrinkled, membranous. Aerial mycelium scant, white. Soluble pigment deep brown.

Starch agar: Growth spreading, colorless. Aerial mycelium abundant, white. Hydrolysis.

Egg medium: Growth extensive, wrinkled, bright yellow. Considerable liquefaction.

Blood agar: Growth in uniformly striated colorless bands; occasional round colonies at margin. Hemolysis positive.

Potato: Growth moist, colorless. Aerial mycelium scant, white at top of plug.

Gelatin: Dull white flakes sinking to bottom as medium liquefies. Rapid liquefaction.

Milk: Coagulation followed by peptonization.

Source: Human spleen in a case of splenic anemia. No record concerning actual pathogenicity.

28a. *Streptomyces bellus* Margalith and Beretta (Margalith, P. and Beretta, G. Mycopathol. Mycol. Appl. **12:** 189–195, 1960).

Morphology: Sporophores long, straight, flexuous with short, open hooks.

Sucrose nitrate agar: Substrate growth light cherry-pink. Aerial mycelium pinkish-white. Soluble pigment light pink.

Glucose-asparagine agar: Growth colorless to light pink with orange tinge. Aerial mycelium white, with small amount of bluish spores. Soluble pigment light pink with orange tinge.

Calcium malate agar: Substrate growth pink, with pinkish-violet reverse. Sporulation abundant, pinkish-blue. Soluble pigment pinkish-violet.

Nutrient agar: Substrate growth hyaline with brownish reverse. Aerial mycelium slight or absent. Soluble pigment brown.

Starch agar: Growth hyaline with colorless to pale pink reverse. Aerial mycelium limited, with bluish-green spores. Starch hydrolyzed.

Glucose-casein digest-yeast-beef agar: Growth pink. Aerial mycelium pinkish with bluish-green spores. Soluble pigment light pink.

Glucose-yeast extract-beef-peptone agar: Growth pink. Aerial mycelium pinkish-white with bluish-green spores. Soluble pigment reddish-brown.

Potato: Growth rough, colorless to brown. No aerial mycelium. Soluble pigment brown.

Gelatin: Slow liquefaction. Soluble pigment brown.

Milk: Growth in form of brownish ring.

Nitrate reduction: None.

Cellulose: Moderate growth.

Carbon utilization: Most sugars readily utilized. Xylose, inulin, and dulcitol not utilized in solid media. Succinate, citrate and glycine not utilized.

Antagonistic properties: Produces antibiotic matamycin, active upon gram-positive bacteria.

Habitat: Soil in Italy.

29. *Streptomyces bikiniensis* Johnstone and Waksman, 1948 (Johnstone, D. B. and Waksman, S. A. J. Bacteriol. **55:** 317–326, 1948).

Morphology: Sporophores straight. Spores oval (Fig. 33).

Sucrose nitrate agar: Growth white, becoming pallid neutral gray with white tinge. Aerial mycelium abundant, white to gray. Soluble pigment light brown. Superficial droplets amber-colored.

Glucose-asparagine agar: Growth abundant. Aerial mycelium white to mouse-gray. Soluble pigment light amber.

Nutrient agar: Growth luxuriant. Aerial mycelium moderate, white. Soluble pigment deep brown.

Starch agar: Growth abundant. Aerial mycelium white, becoming gray. Slight hydrolysis.

Potato: Growth wrinkled and raised, pale ochraceous buff. Soluble pigment brown to black.

Gelatin: Slight liquefaction.

Milk: Surface growth patchy, white. Aerial mycelium gray. Gradual peptonization.

FIGURE 33. *S. bikiniensis*, grown on casein digest-beef extract agar for 12 days, × 3,000–4,000 (Courtesy of K. L. Jones).

Production of H_2S: Positive.

Antagonistic properties: Strongly antagonistic. Produces streptomycin.

Source: Soil from Bikini Atoll.

Type culture: IMRU 3514.

30. *Streptomyces blastmyceticus* Watanabe *et al.*, 1957 (Watanabe, K., Tanaka, T., Fukuhara, K., Miyairi, N., Yonehara, H., and Umezawa, H. J. Antibiotics (Japan) **10A:** 39–45, 1957).

Morphology: Sporophores straight. Spores oval to spherical, 1 by 1.5 μ.

Sucrose nitrate agar: Growth weak, colorless or white. Aerial mycelium poor, white. No soluble pigment.

Glucose-asparagine agar: Growth good, colorless, later cream-colored. No aerial mycelium. No soluble pigment.

Calcium citrate-glycerol agar: Growth colorless or white, later deep olive-buff.

Aerial mycelium thin, powdery, white or pale yellow to pale olive-buff.

Nutrient agar: Growth white, later cream-colored to light brown. Aerial mycelium poor, white to gray. Soluble pigment brown.

Milk: Growth in the form of ring on surface, cream-colored to brown. Aerial mycelium white. Soluble pigment brown. Rapid peptonization.

Potato plug: Growth gray to olive-gray. Aerial mycelium white. Usually no soluble pigment.

Nitrate reduction: Negative.

Starch: Hydrolyzed.

Carbon utilization: Utilizes glucose, fructose, galactose, or starch. Grows poorly on sucrose, lactose, maltose, or inositol. Does not utilize xylose, arabinose, raffinose, rhamnose, mannitol, sorbitol, dulcitol, or salicin.

Antagonistic properties: Produces an antifungal agent designated as blastmycin.

Remarks: Related to *S. flavochromogenes.*

31. *Streptomyces bobiliae* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 121, 1916; **8:** 100, 1919).

Morphology: Elongated sporophores form a few close spirals of a dextrorse type. No spirals according to Jensen (1930). Spores oval and spherical.

Sucrose nitrate agar: Growth abundant, wrinkled, coral-red becoming deep red. Aerial mycelium scant, white; later absent. No soluble pigment.

Glycerol malate agar: Growth cinnamon-buff. No aerial mycelium.

Glucose-asparagine agar: Growth coral-red. No aerial mycelium.

Nutrient agar: Growth gray, becoming brownish to coral-red. No aerial mycelium. Soluble brown pigment in presence of glycerol (Jensen).

Potato: Growth thin, dry, and wrinkled, yellowish, becoming coral-red. No aerial mycelium. Soluble pigment grayish to black.

Gelatin: Growth cream-colored to orange. Aerial mycelium in the form of occasional patches of white. Rapid liquefaction. Soluble pigment brown. Melanin-positive.

Milk: Dark brown ring. Peptonization without coagulation.

Starch media: Growth wrinkled, coral-red with hyaline margin. Aerial mycelium white. Hydrolysis medium.

Nitrate: Good reduction to nitrite.

Cellulose: No growth in solution. Good growth on plate.

Invertase: Positive.

Production of H_2S: Positive.

Temperature: Optimum 37°C.

Antagonistic properties: Produces pigmented antibiotic cinerubin.

Habitat: Common in soil.

Remarks: *S. purpurascens* is considered by Corbaz *et al.* (1957) as a synonym of *S. bobiliae*, except that the latter no longer produces any aerial mycelium or spores. Krassilnikov (1949) considered this species as a variety of *A. ruber.*

Type culture: IMRU 3310.

32. *Streptomyces bottropensis* Konink. Nederl. Gist et Spirit. (Konink. Nederland. Gist et Spirit. Brit. Pat. 762,736, Dec. 5, 1956).

Morphology: Aerial mycelium ramified, with short, open spirals. Spores cylindrical, elliptical to almost spherical, 1 to 4 by 0.6 to 1.2 μ.

Sucrose nitrate agar: Growth abundant, reddish. Aerial mycelium limited. Soluble pigment brown.

Glucose-asparagine agar: Growth good, yellow. Aerial mycelium limited, white to pale gray. No soluble pigment.

Calcium malate agar: Growth good, yellowish-brown. Aerial mycelium white-gray. No soluble pigment.

Starch agar: Growth at first pink, later darker (pH sensitive; acid–pink, alkaline–blue). Aerial mycelium limited, white to gray. Starch hydrolyzed.

Glucose nutrient agar: Growth folded,

yellowish. Aerial mycelium abundant, white. No soluble pigment.

Glucose-yeast extract-peptone agar: Growth yellowish to buff. Aerial mycelium white to gray. No soluble pigment.

Potato agar: Growth smooth, yellowish-brown. Aerial mycelium white to bluish-gray. Soluble pigment at first absent, later dark.

Gelatin: Growth on surface good. Aerial mycelium white. Rapid liquefaction. Soluble pigment dark brown.

Potato: Growth folded, brown to black. No aerial mycelium.

Milk: Growth moderate. No coagulation; no peptonization.

Antagonistic properties: Produces antibiotic B-mycin, active against cocci, gram-positive bacteria, and mycobacteria.

Habitat: Soil.

Remarks: This is one of the organisms that can be either melanin-negative (nutrient agar, yeast extract agar) or melanin-positive (gelatin, potato agar).

33. *Streptomyces brasiliensis* (Spencer, 1921) Waksman (Spencer, E. R. Botan. Gaz. **72:** 285–287, 1921).

Morphology: Aerial mycelium forms straight, branched sporophores. Spores borne in chains on free ends of hyphae, oblong, 1.6. by 0.8 μ.

Sucrose nitrate agar: Growth at first white; after 10 days pale pinkish-buff. Aerial mycelium white and dense. No soluble pigment.

Glucose-asparagine agar: Growth luxuriant, color same as on sucrose nitrate agar, thallus conspicuously zonated. Aerial mycelium powdery, white to pale pinkish-buff. No soluble pigment.

Glycerol malate agar: Growth spreading and not zonated, bordered by submerged mycelial bands of varying width, pearl-white. Aerial mycelium short, loose, and pearl-white.

Potato: Growth vigorous, crumpled, pale pinkish-buff. Aerial mycelium abundant, at first white, later pale pinkish-buff. No soluble pigment. Melanin-negative.

Nut plugs: Growth vigorous, pale pinkish-buff. Aerial mycelium powdery, white. Medium not completely destroyed, but much shrunken and blackened.

Gelatin: Rapid liquefaction. No soluble pigment.

Milk: Rapid coagulation and peptonization.

Habitat: Parasitic on kernels of Brazil nuts.

Remarks: This *Streptomyces* species is to be distinguished from *Nocardia brasiliensis*, a pathogenic organism.

34. *Streptomyces cacaoi* (Waksman, 1932) Waksman and Henrici, 1948 (Waksman, S. A. *In* Bunting, R. H. Ann. Appl. Biol. **19:** 515–517, 1932).

Morphology: Sporophores long; spirals long and open, not compact.

Sucrose nitrate agar: Growth thin, yellowish, later turning reddish-brown. Aerial mycelium light gray to mouse-gray, with white edge. No soluble pigment.

Nutrient agar: Growth brown, covered with tiny patches of ivory-colored aerial mycelium.

Potato: Growth abundant, brownish. Aerial mycelium white to mouse-gray. Melanin-negative.

Gelatin: Growth flocculent. No aerial mycelium. Liquefaction rapid. No soluble pigment.

Nitrate reduction: Limited.

Production of H_2S: Negative.

Antagonistic properties: Certain strains produce an antibiotic designated as cacaomycetin.

Source: Three strains were isolated from cacao beans in Nigeria. They showed slight differences, the foregoing description being based on one strain.

Remarks: Strong proteolytic enzymes, strong diastatic action, no sugar or dextrin

being left in 1 per cent starch solution after a few days.

Type culture: IMRU 3082.

35. *Streptomyces caelestis* DeBoer *et al.*, 1959 (DeBoer, C., Dietz, A., and Hoeksema, H. Canad. Pat. 572,779, March 24, 1959).

Morphology: Sporophores loosely coiled. Spores spherical to oval.

Sucrose nitrate agar: Growth good. Aerial mycelium gray-white. Soluble pigment yellow.

Nutrient agar: Growth fair to good. Aerial mycelium slight pink-white. Soluble pigment brown-tan.

Casein digest-beef agar: Growth good. Aerial mycelium pale glaucous blue. Soluble pigment brown-tan.

Starch agar: No growth.

Tyrosine agar: No growth. No aerial mycelium. No soluble pigment.

Potato: Growth good. Aerial mycelium grayish to blue-white. Soluble pigment brown.

Gelatin: Growth good. Aerial mycelium blue-gray. Soluble pigment brown. Medium liquefaction.

Milk: Growth fair. No soluble pigment. No peptonization.

Nitrate reduction: Negative.

Production of H_2S: Positive.

Carbon utilization: Utilizes a variety of sugars, *dl*-inositol, acetate; limited utilization of starch, glycerol, citrate, and succinate; does not utilize dulcitol, mannitol, inulin, sorbitol, and various other organic acids.

Antagonistic properties: Produces antibiotic celesticetin.

Habitat: Soil in Utah.

Remarks: Similar to *S. glaucus* and *S. chartreusis*.

36. *Streptomyces caeruleus* (Baldacci) Waksman (Baldacci, E. Atti ist. botan. univ. Pavia **3:** 180–184, 1944).

Morphology: Sporophores long, straight, branched, not forming any spirals. Spores cylindrical 1.0 to 1.4 by 2.0 to 2.1 μ.

Agar media: Substrate growth colorless. Aerial mycelium pigmented, at first white, later becoming blue, and finally dark. Soluble pigment grayish-green.

Glycerol agar: Grows slowly; light blue in color.

Carrot agar: Growth at first white; later becoming blue. Aerial mycelium blue, becoming gradually deep blue, and finally dark blue.

Oatmeal agar: Color of growth at first white and aerial mycelium blue, gradually becoming darker in color. The agar is pigmented grayish-green.

Gelatin: Growth slow, grayish-blue. Either no liquefaction or only slow liquefaction.

Milk: Weak growth.

Starch media: Weak greenish growth. Bluish-green pigmentation.

Temperature: Range between 18 and 30°C. Optimum 24°C.

Reaction: Optimum pH 8 to 10.

Cellulose: Not utilized.

Antagonistic properties: Produces antibiotic caerulomycin.

Habitat: Corn straw and decomposing rice straw.

Remarks: Related to *S. violaceoruber*, *S. violaceus*, and *S. viridis*. According to Taber (1959) the distinctive characteristics of a culture that he isolated and identified as *S. caeruleus* are: production of a blue to red indicator pigment; requirement of a neutral or alkaline reaction for growth and production of oblong to cylindrical spores in straight and flexuous chains. It was not chromogenic on peptone media but produced H_2S on iron-peptone agar. It did not grow on unbuffered potato or carrot plugs, litmus milk, and certain synthetic agar media. The culture readily utilized glucose, fructose, galactose, mannitol, sucrose, xylose, starch, and maltose, but did not utilize, or utilized

to a limited extent, mannose, *i*-inositol, adonitol, lactose, ribose, raffinose, and cellulose.

Type culture: IMRU 3798.

37. *Streptomyces caespitosus* Sugawara and Hata, 1956 (Sugawara, R. and Hata, T. J. Antibiotics (Japan) **9A:** 147–151, 1956).

Morphology: Primary verticils produced. Spores oval, 1.3 to 0.5 by 0.3 to 0.5 μ.

Sucrose nitrate agar: Growth hyaline, colorless to faint yellowish-brown. Aerial mycelium white to yellowish-gray to greenish-yellow. Soluble pigment faint yellow.

Calcium malate agar: Growth colorless with yellow-brownish center, changing to dark greenish-yellow to dull yellow. Aerial mycelium white, with yellowish tinge, greenish-yellow at the margin. Soluble pigment faint yellow, pinkish shade in some cultures.

Glucose-peptone agar: Growth humid, wrinkled, cracked in the center; colorless, becoming greenish-yellow-gray, reddish-gray, to dark gray. Aerial mycelium thin, gray. Soluble pigment reddish-brown.

Starch agar: Growth scanty, colorless to faint yellowish-brown, or yellow to orange-yellow. Aerial mycelium cottony, white, cream with lavender patch in the center, becoming yellowish-gray. Soluble pigment faint yellowish-brown.

Nutrient agar: Growth yellow-brown to gray to dark gray. Aerial mycelium gray.

Potato: Growth cream to brownish, center light greenish-yellow. Aerial mycelium white to grayish or gray with pale olive tinge. Soluble pigment absent, or dark brown, or grayish-brown.

Milk: Surface ring yellow to pale yellowish-brown. No aerial mycelium. Soluble pigment pale brown.

Gelatin: Growth cream-colored turning greenish-yellow to reddish-yellow. Aerial mycelium white to yellow. Soluble pigment yellowish-brown. Rapid liquefaction.

Tyrosinase reaction: None.

Nitrate reduction: Positive.

Starch: Hydrolysis.

Carbon utilization: Utilizes various carbohydrates; does not utilize xylose, rhamnose, raffinose, arabinose, mannitol, salicin, dulcitol, inulin, acetate, and succinate.

Antagonistic properties: Produces antibiotic mitomycin, active upon certain neoplasms.

Remarks: Closely related to *S. kitasatoensis* and *S. hachijoensis*.

38. *Streptomyces caiusiae* Dhala *et al.*, 1957 (Dhala, S. A., Poonawalla, F. M., and Bhatnagar, S. S. J. Sci. & Ind. Res. **16C:** 76–80, 1957).

Morphology: Aerial hyphae short and straight; frequently clusters are produced, subdividing at the distal portions into chains of spores. No spirals formed either in synthetic or nonsynthetic media; often tips of the aerial hyphae slightly curved. Spores round to oval, 0.6 to 1.4 by 0.4 to 0.8 μ.

Sucrose nitrate agar: Colonies round, convex, tough, with smooth surface when unsporulated; citron-yellow, later turning brown. Aerial mycelium white, turning yellowish, then gray. Soluble pigment at first yellow but later darkened to a brown tinge.

Glucose-asparagine agar: Growth citron-yellow. Aerial mycelium white. Soluble pigment yellowish-brown.

Nutrient agar: Growth wrinkled, with irregular margins and radial ridges in old cultures. White aerial mycelium.

Starch agar: Colonies smooth, colorless after 2 days; on further incubation, they become large and wrinkled with radiating ridges. Aerial mycelium white, turning gray. Soluble pigment brown. Starch weakly hydrolyzed.

Potato: Growth luxuriant, citron-yellow. Aerial mycelium white, turning gray. Plug turns black.

Milk: Pellicle produced. Coloration of milk brownish to black. Peptonization positive.

Gelatin: Sediment buff-colored. Liquefaction medium.

Tyrosinase reaction: Positive.

Nitrate reduction: None.

Carbon sources: Sugars readily utilized, with the exception of acetate, benzoate, cellulose, dulcitol, *i*-inositol, and salicylate.

Antagonistic properties: Active primarily upon gram-negative bacteria and fungi.

Remarks: Closely related to *S. antibioticus*.

39. *Streptomyces californicus* (Waksman and Curtis, 1916) Waksman and Henrici (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 22, 1916; Waksman, S. A. *ibid.* **8:** 104, 1919).

Synonyms:

Streptomyces puniceus Finlay and Sobin, 1950.

Streptomyces vinaceus Mayer *et al.*, 1951.

Streptomyces floridae Bartz *et al.*, 1951.

Streptomyces griseus var. *purpureus* Burkholder *et al.*, 1955.

Streptomyces purpureus (Burkholder, 1955) Waksman, 1959.

Morphology: The original culture was reported to form sporophores with long, narrow, open sinistrorse spirals. According to Okami, however, the sporophores are straight. Recent examinations of the original culture of Waksman and Curtis (Burkholder *et al.*, 1955) did not reveal any spirals either.

Sucrose nitrate agar: Growth spreading, vinaceous-colored. Aerial mycelium powdery, light neutral gray to ash-gray. No soluble pigment.

Glucose-asparagine agar: Growth restricted, much folded, cream-colored, with sulfur-yellow tinge.

Nutrient agar: Growth thin, restricted, yellowish to cream-colored, Melanin-negative.

Starch agar: Growth spreading, pink center with colorless to gray margin. Hydrolysis rapid.

Potato: Growth glossy, yellow to red, turning red-brown.

Gelatin: Growth gray, moist, abundant. No soluble pigment. Liquefaction medium.

Milk: Surface growth faint, brownish. Coagulation and slow peptonization.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Cellulose: Growth scant but definite.

Carbon utilization: According to Burkholder, the various strains utilize D-xylose, D-glucose, D-galactose, D-fructose, cellobiose, D-maltose, D-mannitol, and starch. Growth poor with L-arabinose, L-rhamnose, D-lactose, sucrose, D-raffinose, dulcitol, *i*-inositol, and salicin.

Temperature: Optimum 37°C.

Invertase: Positive.

Antagonistic properties: Routien and Hofmann (1951) first demonstrated that cultures of *S. californicus* are capable of producing viomycin. The same antibiotic was found to be produced by other strains of this organism.

Habitat: Soil.

Remarks: Waksman and Curtis reported the production of spirals in their original description of *S. californicus* (see also Waksman, 1919). Several authors who studied the *S. californicus* culture more recently described the aerial mycelium as straight to wavy to strongly flexuous (Burkholder *et al.*, 1955; Kutzner, 1956; Ettlinger *et al.*, 1958). Since the other properties of the now-available *S. californicus* fit with the original description, it might be assumed that the strongly flexuous aerial hyphae were considered as spirals originally. In 1955 Burkholder *et al.* made a comparative study of several viomycin-producing organisms which were originally described under the names *S. floridae*, *S. puniceus*, *S. vinaceus*, *S. californicus*, and several others. All these cultures behaved in a similar manner, with only minor differences between this group and *S. californicus* ATCC 3312; namely,

"with the exception of ATCC 3312, all isolates liquefy gelatin rapidly and produce viomycin or similar antibiotic compounds." No studies seem to have been made, however, of antibiotics produced by *S. californicus* ATCC 3312. Only because the original description of *S. californicus* gave spiral formation and these organisms did not, the several viomycin-producing organisms and the *S. californicus* ATCC 3312 were described as a variety of *S. griseus*, namely *S. griseus* var. *purpureus*. This was due to the fact that *S. griseus* had the same morphology and color of the aerial mycelium, and because several streptomycin-producing strains are known to form also a red-gray color in the substrate growth.

Type culture: IMRU (ATCC) 3312.

40. *Streptomyces calvus* Backus *et al.*, 1957 (Backus, E. J., Tresner, H. D., and Campbell, T. H. Antibiotics & Chemotherapy **7:** 532–541, 1957).

Morphology: Sporophores form short loose spirals. Spores globose to elongated, 0.6 to 1.0 by 1.0 to 1.8 μ (Fig. 34).

Sucrose nitrate agar: Growth cream-colored to yellowish. Aerial mycelium scanty, white to gray.

Glucose-asparagine agar: Growth ivory-yellow. Aerial mycelium scanty, white.

Calcium malate agar: Growth colorless to yellow. Aerial mycelium scanty white to gray. Crystalline pellets formed in growth zones.

Starch agar: Growth colorless to yellowish. Aerial mycelium white to mouse-gray.

Nutrient agar: Substrate growth light yellow. Aerial mycelium scanty, white. No soluble pigment. Melanin-negative.

Potato plug: Growth gray. Aerial mycelium scanty, white to light gray. Plug discolored.

Gelatin: Growth colorless to yellow. No aerial mycelium. Partial liquefaction. No soluble pigment.

Milk: Growth colorless to yellow. Coagulation and moderate peptonization.

Cellulose: Growth yellow. No decomposition of cellulose.

Production of H_2S: Negative.

Carbon utilization: *d*-fructose, *i*-inositol, lactose, *d*-mannitol, *d*-raffinose, *l*-rhamnose, sucrose, *d*-trehalose, and *d*(+)-xylose readily utilized; *l*-arabinose, *d*-melibiose, and salicin utilized poorly; dextrin, esculin, demelezitose, and adonitol not utilized at all.

Antagonistic properties: Produces nucleocidin, an antibiotic possessing antitrypanosomal properties.

Habitat: Soil in India.

Remarks: This organism is closely related to *S. annulatus*.

41. *Streptomyces candidus* (Krassilnikov, 1941) Waksman (Not *Streptothrix candida* Petruschky). (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 49, 1941).

Morphology: Sporophores long, straight or wavy, but never forming spirals; occasionally arranged in broom-shaped bodies or fascicles. Spores oblong to cylindrical ("fragmentation spores"), 1.0 to 2.0 by 0.6 to 0.8 μ.

Sucrose nitrate agar: Growth colorless. Aerial mycelium velvety, white. No soluble pigment.

Nutrient agar: Growth good, lichenoid or smooth. Aerial mycelium whitish. Melanin-negative.

Gelatin: Slow liquefaction. Melanin-negative.

Potato: Growth colorless, lichenoid. Aerial mycelium poorly developed. No soluble pigment or brownish.

Milk: No coagulation; good peptonization.

Starch: Rapid hydrolysis.

Cellulose: Good growth.

Nitrate reduction: Positive.

Sucrose: Inversion.

Production of H_2S: Negative.

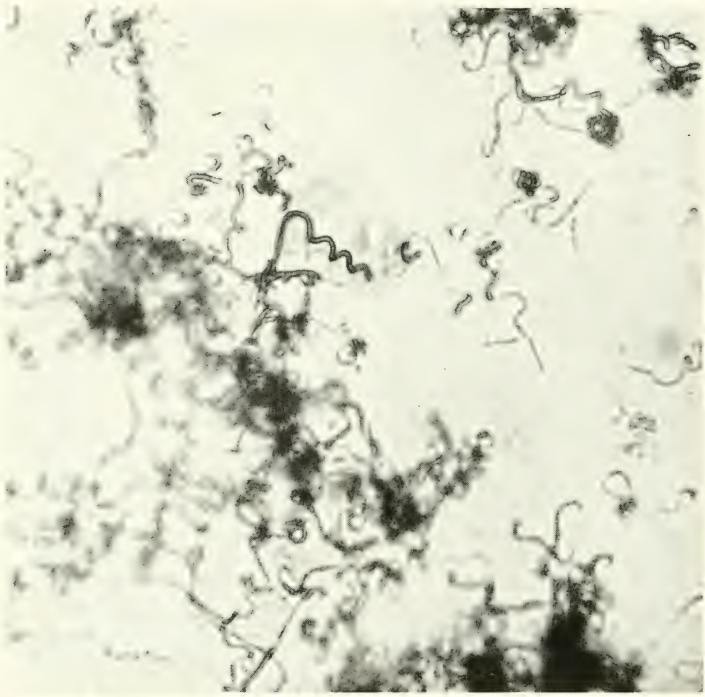

FIGURE 34. Sporophores of *S. calvus* (Reproduced from: Backus, E. J. *et al.* Antibiotics & Chemotherapy **7:** 536, 1957).

Antagonistic properties: Weak.

Habitat: Soil.

Remarks: Certain varieties of this species have also been described. This is true, for example, of *A. fasciculus*, which Krassilnikov himself considered as a variety of *S. candidus*; it is also true of *A. farinosus* Krassilnikov and of *A. candidus* var. *alboroseus* described by Gause *et al.* (1957). *S. nitrosporeus* Okami (1952) appears to be closely related, if not identical to it. Ettlinger *et al.* (1958) consider this organism as related to *S. griseus*.

Type culture: IMRU 3416.

42. *Streptomyces canescus* Hickey *et al.*, 1952 (Hickey, R. J., Corum, C. J., Hidy, P. H., Cohen, I. R., Nager, U. F. B., and Kropp, E. Antibiotics & Chemotherapy **2:** 472–483, 1952).

Morphology: Sporophores straight or curved, not forming any spirals. Spores globose, 1.0 to 1.3 by 1.3 to 2.6 μ (Fig. 35).

Calcium malate agar: Growth gray to rose-gray; reverse yellow to tan. No soluble pigment.

Yeast extract-casein digest agar: Growth effuse to convex, edge filamentous; reverse brown. Aerial mycelium powdery, varying from gray-white to gray. No soluble pigment.

Acid-glucose-peptone agar: Growth at first white, then tan. Aerial mycelium faintly

greenish, produced after 14 days. Amber pigment diffused throughout medium.

Egg medium: Growth tan, wrinkled. No sporulation after 10 days; limited white sporulation observed in 14 days. Soluble pigment brown. Very slow liquefaction after 28 days.

Potato: Growth light gray, wrinkled. Soluble pigment deep brown.

Gelatin: Liquefaction rapid. Soluble pigment deep brown.

Milk: Soft, rennet curd formed at 36°C after 48 hours; completely peptonized in 12 days.

Starch: Hydrolysis strong.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Temperature: Optimum 36°C.

Carbon utilization: Utilizes glucose, arabinose, trehalose, xylose, sucrose, maltose, galactose, dextrin, soluble starch, mannitol, glycerol, and salicin. No growth with sor-

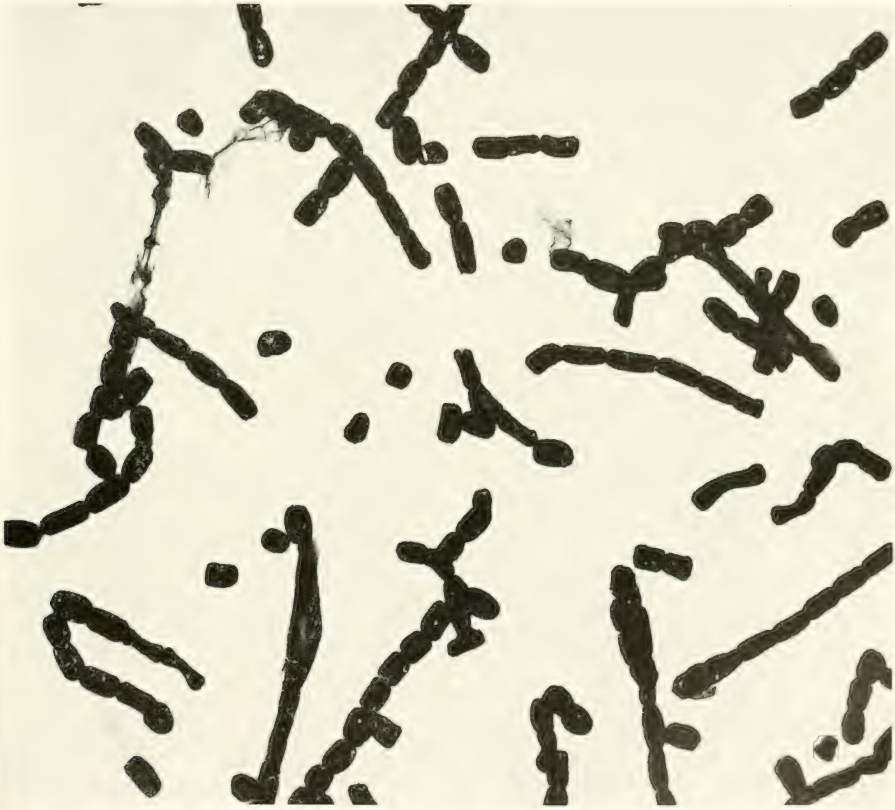

FIGURE 35. *S. canescus*, grown on casein digest-beef extract agar for 12 days. × 3,000–4,000 (Courtesy of K. L. Jones.)

bose, melezitose, dulcitol, rhamnose, sorbitol, melibiose, phenol, raffinose, and lactose.

Antagonistic properties: Produces antifungal antibiotic ascosin.

Source: Contaminated fungus plate.

Remarks: This species is now included with *S. coelicolor* (Kutzner and Waksman, 1959).

Type culture: IMRU 3782; NRRL 2419.

43. *Streptomyces canus* Heinemann *et al.*, 1953 (Heinemann, B., Kaplan, M. A., Muir, R. D., and Hooper, I. R. Antibiotics & Chemotherapy **3:** 1239–1242, 1953).

Morphology: Aerial mycelium forms numerous loosely wound spirals. Spores spheroidal, 1.0 to 1.2 by 1.6 to 1.8 μ.

Sucrose nitrate agar: Growth moderate, wrinkled, yellow-brown. Aerial mycelium scant. No soluble pigment.

Glycerol-asparagine agar: Growth abundant, cream-colored, turning russet-brown with aging. Aerial mycelium abundant, slate-gray. Soluble pigment amber.

Calcium malate agar: Growth moderate, golden colored. Aerial mycelium scant. No soluble pigment.

Nutrient agar: Growth abundant, yellow. Aerial mycelium white to light yellow. Soluble pigment faint yellow.

Potato: Growth abundant, cream-colored. No aerial mycelium. Slight reddish-brown darkening of the potato. Melanin-negative.

Gelatin: Moderate liquefaction at 26°C in 14 days. No soluble pigment.

Milk: Alkaline with no coagulation; slight peptonization in 14 days.

Starch: Hydrolysis in 96 hours at 30°C.

Nitrate reduction: Positive in 96 hours at 30°C.

Carbon utilization: Good growth with arabinose, rhamnose, xylose, dextrose, galactose, fructose, cellobiose, lactose, maltose, sucrose, dextrin, inulin, raffinose, soluble starch, glycerol, inositol, mannitol, and sodium salicylate. No growth observed with dulcitol, sorbitol, sodium acetate, sodium citrate, sodium formate, sodium malate, sodium oxalate, sodium tartrate, or sodium succinate.

Antagonistic properties: Produces the antibiotic amphomycin, active against gram-positive bacteria.

Habitat: Soil.

Type culture: ATCC 12,237.

44. *Streptomyces carnosus* (Millard and Burr, 1926) Waksman and Henrici (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Spores cylindrical, 1.0 by 0.75 μ.

Sucrose nitrate agar: Growth pale smoky-gray to olive-gray. Aerial mycelium abundant, gray. Colorless guttation drops appear over the whole surface. Soluble pigment ivory-yellow to cartridge-buff.

Potato: Growth lichenoid. Aerial mycelium gray to brownish with white spots. Plug becomes colored gray to black.

Gelatin: Surface growth. Aerial mycelium white in center, gray at margin. Rapid liquefaction. Soluble brown pigment.

Milk: Surface growth good. No aerial mycelium. Positive coagulation and peptonization.

Starch: Hydrolysis.

Tyrosinase reaction: Negative.

Nitrate reduction: Positive.

Habitat: Potato scab.

45. *Streptomyces catenulae* Davisson and Finlay, 1959 (Davisson, J. W. and Finlay, A. C. U. S. Pat. 2,895,876, July 21, 1959).

Morphology: Growth wrinkled with smooth edge. Sporophores in form of short clusters; a few tight spirals. Spores oval to cylindrical, 1.0 by 1.3 μ.

Agar media: Growth dark brown to dark greenish-brown. Aerial mycelium dark olive-gray to brown.

Sucrose nitrate agar: Growth transparent, white. Aerial mycelium light gray. No soluble pigment.

Calcium malate agar: Growth poor. Aerial mycelium mouse-gray, with some white. Calcium malate digested.

Nutrient agar: Growth pale yellow. Aerial mycelium white, turning pale gray. Soluble pigment pale yellow. Nonchromogenic.

Glucose-yeast extract-beef-peptone agar: Growth dark brown. Aerial mycelium olive-gray with white. Soluble pigment medium brown.

Starch agar: Growth brownish-orange. Aerial mycelium poor, mouse-gray. Good hydrolysis.

Gelatin: Growth moderate. Aerial mycelium buff, some white. No soluble pigment. Poor liquefaction.

Potato: Growth good, greenish. Aerial mycelium pale olive to smoke-gray to brown. Soluble pigment dark greenish or absent.

Nitrate reduction: None.

Antagonistic properties: Produces antibiotic catenulin.

Habitat: Soil.

Type culture: ATCC 12,476.

46. *Streptomyces cavourensis* Giolitti, 1958 (Giolitti, G. Belgian Pat. 560,930, March 18, 1958*).

Morphology: Aerial mycelium produces spirals on certain media. Spores spherical to elliptical.

Sucrose nitrate agar: Growth yellowish. Aerial mycelium chalky white to yellowish. No soluble pigment.

Glycerol-asparagine agar: Growth hazel-colored. Aerial mycelium whitish. Soluble pigment faint brown.

Calcium malate agar: Growth scanty, yellow-brownish. Aerial mycelium scanty, white. Soluble pigment scarce, yellow-brownish.

Nutrient agar: Growth orange-brown. Aerial mycelium scanty, chalky white to yellowish. Soluble pigment light brown.

Glucose agar: Growth brown, wrinkled. Aerial mycelium white-yellowish. Soluble pigment dark brown.

Potato agar: Growth dark brown. Aerial mycelium gray with dark yellow dots. Soluble pigment brown.

Oatmeal agar: Growth light brown, wrinkled. Aerial mycelium gray with brown patches. Soluble pigment light brown.

Starch agar: Growth scanty, yellow-brownish. Aerial mycelium scanty, white-grayish. Soluble pigment brownish. Strong hydrolysis.

Gelatin: Growth scanty, brown, wrinkled. Aerial mycelium scanty, gray. Soluble pigment brownish. Liquefaction fairly good.

Potato: Growth brown with yellow edges, wrinkled. Aerial mycelium grayish. Soluble pigment brown.

Milk: Growth consists of white to yellow ring around surface. Positive coagulation and peptonization.

Nitrate reduction: Negative.

Antagonistic properties: Produces flavensomycin, an antibiotic active against filamentous and yeast-like fungi, and to a certain extent some gram-positive bacteria. Very active against some insects.

Type culture: IMRU 3758.

47. *Streptomyces celluloflavus* Nishimura *et al.*, 1953 (Nishimura, H., Kimura, T., and Kuroya, M. J. Antibiotics (Japan) **6A:** 57–65, 1953).

Morphology: Sporophores straight with a few flexible, hooked spirals. Spores nearly spherical, 1.0 by 0.9 μ.

Sucrose nitrate agar: Growth glossy, developing deep into medium, later becoming yellow. Soluble pigment faint sulfur-yellow.

Glycerol malate agar: Growth yellow, later turning white to pale olive-buff with blackish center. Aerial mycelium cottony, white, with grayish patches, later turning olive-buff. Soluble pigment yellow.

Glucose-asparagine agar: Growth cream to yellow. Aerial mycelium scant, cottony,

* Supplemented by personal communication.

white to gray. Soluble pigment sulfur-yellow.

Nutrient agar: Growth olive-buff, turning colorless. Aerial mycelium scant, cottony, white to grayish. Soluble pigment yellow with tinge of green to gold.

Potato: Growth wrinkled, deep olive-buff. Aerial mycelium white to olive-buff. Soluble pigment deep olive-buff.

Gelatin: Growth ivory-yellow to olive-buff on surface of liquefied layer. No aerial mycelium. Faint brownish pigment. Rapid to medium liquefaction.

Milk: Growth yellow to dark olive-buff. Aerial mycelium white. Soluble pigment reddish-brown. Coagulation and rapid peptonization.

Tyrosine medium: Growth ivory-yellow to cream-buff. Aerial mycelium absent or scant white. Soluble pigment greenish-yellow.

Cellulose agar: Growth poor. Soluble pigment yellow.

Production of H_2S: Negative.

Antagonistic properties: Produces thiolutin, aureothricin.

Habitat: Soil.

48. *Streptomyces cellulosae* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 683–688, 1914).

Description after Jensen, H. L. Soil Sci. **30:** 65, 1930.

Morphology: Sporophores straight; no spiral formation. Spores almost spherical, 1.3 μ in diameter.

Sucrose nitrate agar: Growth at first transparent, becoming lemon-yellow. Aerial mycelium light gray, later deep slate-gray. Soluble pigment may be lemon-yellow.

Calcium malate agar: Colonies yellowish; aerial mycelium gray to white-gray. Soluble pigment yellow.

Glucose-asparagine agar: Growth abundant; aerial mycelium gray. Soluble yellow pigment, especially with high nitrogen concentration.

Glucose nutrient agar: Good substrate growth, at first cream-colored, later sulfur-yellow. Aerial mycelium white, later gray.

Potato: Growth light cream-colored, later often yellow. Aerial mycelium white, later slate-gray.

Gelatin: Growth yellowish-gray to grayish-black. Rapid liquefaction.

Milk: Rapid coagulation and peptonization.

Cellulose: Growth good.

Esculin: Hydrolysis.

Starch: Diastatic action strong.

Nitrate: Reduction weak.

Invertase: Negative.

Production of H_2S: Negative.

Temperature: Optimum 30–35°C.

Pigment: Soluble in alcohol and other organic solvents.

Antagonistic properties: Produces antibiotics fungichromin and actinomycin.

Habitat: Very common in soil.

Remarks: The culture described by Krainsky (1914) produced an aerial mycelium of a gray color ("like *diastaticus*"). Later, however, Krainsky's culture was found to produce a grayish-yellow aerial mycelium like the streptomycin-producing *S. griseus* (Ettlinger *et al.*, 1958); these authors also consider the *S. cellulosae* strains obtained from ATCC and NRRL as closely related to *S. griseus*. Most probably, one of these cultures was used in the studies of Tresner and Danga (1958), who mention a yellow-cream-buff color of the aerial mycelium. In contrast to these *cellulosae* strains now available for comparison, Jensen (1930a) described five soil isolates as *A. cellulosae* with a distinctive slate-gray aerial mycelium, in agreement with Krainsky's description. Krassilnikov (1949) considers this organism as a variety of *A. flavus*.

Type culture: IMRU 3313, 3780.

49. *Streptomyces chartreusis* Calhoun and Johnson, 1956 (Calhoun, K. M. and John-

son, L. E. Antibiotics & Chemotherapy **6:** 294–298, 1956).

Morphology: Aerial hyphae branch profusely producing closed or open spirals, sinistrorse and dextrorse, depending on composition of medium. Spiral chains consist of 3 to 7 turns. Spores powdery, blue-gray to blue-green, depending on the medium. Most spirals occur singly; a few are found in groups of two or three measuring 5 to 20 μ in length and 3 to 5 μ in width. Spores spherical to oval, 1.0 to 1.5 μ in diameter.

Sucrose nitrate agar: Growth profuse, raised and somewhat wrinkled, honey-colored. Aerial mycelium of young colonies white to pale gray; older colonies have blue center.

Glucose-asparagine agar: Growth profuse, blue-gray. Soluble pigment yellow.

Starch agar: Growth profuse. Center of colonies blue-green, edges white. No soluble pigment. Good hydrolysis.

Nutrient agar: Growth profuse. Center of colonies blue-gray, edges powdery white. No soluble pigment.

Potato: Growth light, raised with some wrinkling. Center of colonies blue-gray with white edges. No soluble pigment.

Gelatin: Growth blue-green in center with white edges. Soluble pigment yellow-green to black. Slow liquefaction. Melanin-positive.

Milk: Growth moderate, blue-gray and white. Slow peptonization.

Nitrate broth: Blue-green ring and white pellicle. Soluble pigment yellow-tan. Nitrate strongly reduced.

Production of H_2S: Positive.

Antagonistic properties: Produces antibiotic chartreusin.

Remarks: Ettlinger *et al.* (1958) considered this species to be related to *S. viridochromogenes.*

50. *Streptomyces chibaensis* Suzuki *et al.*, 1958 (Suzuki, S., Nakamura, G., Okuma, K., and Tomiyama, Y. J. Antibiotics (Japan) **11A:** 81–83, 1958).

Morphology: Sporophores wavy, producing numerous small spirals.

Sucrose nitrate agar: Growth slow, penetrating into medium, yellow reverse. At first no aerial mycelium; later mycelium produced, white becoming gray, then reddish-gray or black-buff. Soluble pigment slight yellow.

Nutrient agar: Growth good. Reverse cream-colored or yellow. Aerial mycelium powdery, white. Soluble pigment absent or slightly yellow.

Glucose-asparagine agar: Growth good, yellow, later becoming brown. Aerial mycelium powdery, white, later becoming dark brown. Soluble pigment yellow.

Starch agar: Growth good, white, later becoming olive-yellow. Strong hydrolysis of starch.

Potato: Growth good, raised. Aerial mycelium white or cream-colored, powdery. No change in color of plug.

Gelatin: Growth on surface and in medium poor. No aerial mycelium. Liquefaction absent or limited. Melanin-negative.

Milk: Growth good; white to cream-colored pellicle. No aerial mycelium. No soluble pigment. Peptonization slow.

Carbon sources: Utilizes readily a variety of carbon sources, but not inulin.

Antagonistic properties: Produces antibiotic cellocidin, active against gram-positive and gram-negative bacteria; this antibiotic also possesses anticancer properties.

Habitat: Soil in Japan.

Remarks: The organism is closely related to *S. flavus.*

51. *Streptomyces chrysomallus* Lindenbein, 1952 (Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Substrate growth soft, consisting of long, branching hyphae, with numerous staining granules. Sporophores long,

straight; no spirals. Spores oval to elliptical; surface smooth.

Glycerol nitrate agar: Growth light yellow. Aerial mycelium powdery, white. Soluble pigment golden yellow.

Glucose-asparagine agar: Growth smooth, colorless to yellowish. Aerial mycelium powdery, white. Soluble pigment faint yellow.

Glycerol malate agar: Growth thin, smooth, colorless to light yellow. Aerial mycelium powdery, grayish-white.

Nutrient agar: Growth poor, shiny, golden yellow. Aerial mycelium white, powdery. Soluble pigment golden yellow. Melanin-negative.

Glucose-peptone agar: Growth yellowish with tinge of orange. Aerial mycelium grayish-white. Soluble pigment light yellow to golden yellow.

Starch-casein agar: Growth colorless, with yellowish reverse. Aerial mycelium powdery, chalk-white. Strong hydrolysis of starch. No soluble pigment.

Potato: Growth heavy, yellow, becoming brownish-yellow or orange. Aerial mycelium cottony white to yellowish-white.

Gelatin: Surface growth heavy, light to dark yellow. Aerial mycelium white. Soluble pigment yellow-brown to deep brown, only in liquefied portion. Strong liquefaction. Melanin-negative.

Milk: Growth colorless, with light yellow reverse. Aerial mycelium cottony, snow-white, becoming yellowish. Coagulation slight. Strong peptonization.

Cellulose: Growth very weak.

Production of H_2S: Negative.

Antagonistic properties: Produces actinomycin C; some strains also produce the antifungal cycloheximide.

Habitat: Soil.

Remarks: Ettlinger *et al.* (1958) considered it as a member of the *S. griseus* group. A complete description of this organism was also given by Frommer (1958). Frommer (1959) described a variety of this organism under the name *fumigatus*. It differed from the type species by producing a mouse-gray aerial mycelium on synthetic media, no aerial mycelium on potato and gelatin, and by displaying more limited proteolytic properties.

Type culture: IMRU 3657.

52. *Streptomyces cinereoruber* Corbaz *et al.*, 1957 (Corbaz, R., Ettlinger, L., Keller-Schierlein, W., and Zähner, H. Arch. Mikrobiol. **25:** 325–332, 1957).

Morphology: Sporophores straight; no spirals. Spores slightly elongated, 0.9 to 2 by 0.7 to 1 μ; surface of spores smooth.

Glycerol nitrate agar: Growth thin, light carmine-red, in 7 days dark red. Aerial mycelium ash-gray. Soluble pigment light carmine.

Glucose-asparagine agar: Substrate growth thin, greenish-gray to bluish-gray. Aerial mycelium ash-gray. No soluble pigment.

Gelatin: Surface growth light carmine to light brown. Aerial mycelium light gray. Soluble pigment red-brown. Medium liquefaction. Melanin-positive.

Starch agar: Vegetative growth coral-red. Aerial mycelium ash-gray. Soluble pigment carmine. Hydrolysis limited.

Potato: Growth lichenoid, brownish-yellow. Aerial mycelium ash-gray. Soluble pigment bluish-gray.

Milk: Pellicle light brown with sparse aerial mycelium, powdery, white-gray. Coagulation and peptonization. Reaction turns acid.

Carbon utilization: Utilizes xylose, arabinose, and other sugars. Does not utilize L-rhamnose, D-fructose, raffinose, inulin, *d*-sorbitol.

Antagonistic properties: Produces antibiotic rhodomycin.

Remarks: This organism is closely related to *S. bobiliae* and *S. purpurascens*. *S. cinereoruber* var. *fructofermentans* is a variety,

based on differences in sugar utilization, and produces the antibiotic cinerubin.

53. *Streptomyces cinnamomeus* Benedict *et al.*, 1954 (Benedict, R. G., Dvonch, W., Shotwell, O. L., Pridham, T. G., and Lindenfelser, L. A. Antibiotics & Chemotherapy **2:** 591, 1952; **4:** 1140, 1954).

The correct name of this organism is *S. cinnamomeus* f. *cinnamomeus*.

Morphology: Sporophores straight; later descriptions indicate verticil formation. Spores globose, 0.6 μ (Fig. 36).

Sucrose nitrate agar: Growth colorless to white to cream-colored. Aerial mycelium white to light cinnamon.

Glucose-asparagine agar: Growth colorless; light greenish-yellow to dull yellowish-orange in reverse. Aerial mycelium white to cinnamon.

Nutrient agar: Growth cream-colored to light lemon-yellow. No aerial mycelium. No soluble pigment.

Oatmeal agar: Growth tough, leathery, yellowish-green to cream-yellow. Aerial mycelium floccose, pale violet to faint cinnamon. Exudate tan to white.

Starch agar: Growth colorless to brownish. Aerial mycelium white. Hydrolysis.

Potato: Growth grayish-white to yellow-green to light brown. Aerial mycelium light gray to gray. No soluble pigment.

Gelatin: Growth flocculent, dirty yellow to white. Aerial mycelium cretaceous. No soluble pigment. Rapid liquefaction.

Milk: Ring light brown. Aerial mycelium limited, white. Rapid peptonization.

Carbon utilization: Utilizes xylose, fructose, inositol, starch, dextrin, galactose, and maltose. Does not utilize arabinose, rhamnose, dulcitol, and salicin.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Temperature: Good growth at 25–37°C.

Antagonistic properties: Produces cinnamycin, a polypeptide antibiotic.

Source: Japanese soil.

FIGURE 36. Hyphae of *S. cinnamomeus* showing character of verticils of sporogenous branches (Reproduced from: Duggar, B. M. *et al.* Ann. N. Y. Acad. Sci. **60:** 85, 1954).

Remarks: Pridham *et al.* (1956) described a second form under the name of *S. cinnamomeus* f. *azacoluta*; it produced a shell-pink aerial mycelium on starch agar and an antibiotic, duramycin.

Type culture: IMRU 3664.

54. *Streptomyces cinnamonensis* Okami, 1953 (Okami, Y., Maeda, K., Kosaka, H., Taya, O., and Umezawa, H. Japan. J. Med. Sci. Biol. **6:** 87–90, 1953).

Morphology: Sporophores long, flexible, hooked, but no true spirals. Spores elliptical to oval.

Nutrient agar: Growth colorless to dark. No aerial mycelium. Soluble pigment absent or slightly brown.

Glycerol agar: Growth colorless. Scant white aerial mycelium or white with pale cinnamon-pinkish to light brownish-vinaceous tinge. No soluble pigment.

Glucose-asparagine agar: Growth colorless to light cream-colored. Aerial mycelium white to white-pinkish-cinnamon. No soluble pigment.

Potato: Growth dark to light cream-colored. No aerial mycelium. No soluble pigment; later, black pigment produced around growth.

Gelatin: Growth colorless to dark brownish. Aerial mycelium in form of white patches. Soluble pigment brown. No or very slow liquefaction.

Milk: Growth cream-colored to brownish surface ring. Aerial mycelium absent or scant white. Soluble pigment absent or slightly brown. Coagulation and peptonization absent or very slow.

Starch agar: Growth colorless. Aerial mycelium white with pinkish tinge. No soluble pigment. Hydrolysis good.

Cellulose: No growth.

Nitrate reduction: None.

Production of H_2S: Positive.

Carbon utilization: Sucrose, mannose, dextrin, galactose, glycerol, fructose, glucose, maltose, mannitol, xylose, and sodium succinate utilized. Arabinose, esculin, rhamnose, dulcitol, sodium acetate, inulin, lactose, salicin, and raffinose not utilized.

Antagonistic properties: Produces an antibiotic active against mycobacteria and identical with actithiazic acid or thiozolidone.

Remarks: The culture resembles *S. roseochromogenes* in color of growth and in no or slow liquefaction of gelatin. It differs in the lack of spiral formation and of nitrate reduction. Gause *et al.* (1957) described a variety of this organism under the name of *A. cinnamonensis* var. *proteolyticus*. *A. daghestanicus* and *A. fumanus* described by these authors apparently also belong to this group, although they differ from it in some respects. According to Benedict and Pridham (1959) a group of cooperators considered this organism as *S. cinnamonensis*, *S. virginiae*, *S. acidomyceticus*, *S. roseochromogenes*, and *S. lavendulae*; an opinion was expressed that all of these are probably related to *S. lavendulae*.

Type culture: ATCC 12,308.

55. *Streptomyces circulatus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 60, 1941).

Morphology: Sporophores produce verticils with spiral-shaped short branches. Spores cylindrical or oblong, 1.5 by 0.7 μ, some rounding up with age of culture.

Synthetic agar: Growth good, colorless. Aerial mycelium abundant, white.

Nutrient agar: Growth weak. No aerial mycelium.

Gelatin: Liquefaction weak.

Milk: No coagulation; slow peptonization.

Starch: Hydrolysis weak.

Cellulose: No growth.

Paraffin: Growth good. Aerial mycelium white.

Nitrate reduction: Weak.

Sucrose: No inversion.

Antagonistic properties: Limited.

Habitat: Soil.

56. *Streptomyces citreus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 684, 1914; Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 116, 1916; **8:** 121, 1919). Not *Actinomyces citreus* Gasperini, 1894.

Morphology: Sporophores form long narrow, open spirals, dextrorse. Spores spherical to oval, 1.2 to 1.5 by 1.2 to 1.8 μ.

Sucrose nitrate agar: Growth abundant, raised, wrinkled, citron-yellow. Aerial mycelium white to citron-yellow. No soluble pigment.

Malate-glycerol agar: Growth creamy to yellow. Aerial mycelium white with mouse-gray tinge. No soluble pigment.

Glucose-asparagine agar: Growth glossy, olive-yellow; center elevated. Aerial mycelium white to pinkish. No soluble pigment.

Nutrient agar: Growth restricted, green. No aerial mycelium. No soluble pigment.

Potato: Growth yellowish to gray. Aerial mycelium white. No soluble pigment.

Gelatin: Surface growth restricted, yellow-

ish. Aerial mycelium white. Liquefaction medium. Melanin-negative.

Milk: Surface growth cream-colored. Coagulation followed by rapid peptonization.

Starch media: Growth abundant, citron-yellow to yellowish-green. Aerial mycelium pinkish. Rapid hydrolysis of starch.

Cellulose: No growth.

Invertase: Positive.

Nitrate: Slight reduction to nitrite.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Negative.

Habitat: Garden soil.

Remarks: Since Krainsky's culture was not available for comparison, the above description is based upon that of Waksman and Curtis (1916) and Waksman (1919); some differences exist between this description and that of Krainsky. Ettlinger *et al.* (1958) considered this culture as a strain of *S. griseus*. Krassilnikov (1949) considers this organism as similar to Gasperini's culture, both being looked upon as varieties of *A. flavus*.

Type culture: IMRU 3574.

57. *Streptomyces clavifer* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores long, straight, some terminating in club-shaped structures. Spores cylindrical, 1.5 by 1.0 μ.

Sucrose nitrate agar: Growth gray to brick-red. Aerial mycelium white, sprinkled with cinnamon-drab. Soluble pigment yellowish to brown.

Potato: Growth wrinkled, gray to orange to brown. Aerial mycelium gray to olive-buff. Color of plug gray to brown.

Gelatin: Growth gray to buff. Aerial mycelium white. Medium liquefaction. Soluble pigment yellow to reddish-yellow.

Starch: Hydrolysis.

Tyrosinase reaction: Positive.

Nitrate: No reduction.

Temperature: Fair growth at 37.5°C.

Habitat: Limed soil and common scab of potatoes.

Remarks: Kutzner (1956) described the original culture obtained from CBS as producing an ash-gray aerial mycelium without spirals, and as melanin-negative; this culture was indistinguishable from *S. craterifer* also obtained from CBS. Krassilnikov (1949) considered it as a variety of *A. scabies*.

58. *Streptomyces coelicolor* (Müller, 1908) Waksman and Henrici *emend.* Kutzner and Waksman (Müller, R. Centr. Bakteriol. Parasitenk. Abt. I, Orig. **46:** 195, 1908; Kutzner, H. J. and Waksman, S. A. J. Bacteriol. **78:** 528–538, 1959).

Synonyms:

Streptothrix coelicolor Müller (Müller, 1908).

Actinomyces albidoflavus (strain Höhle, CBS).

Actinomyces alni (strain v. Plotho, CBS).

Streptomyces canescus Hickey *et al.* (Hickey *et al.*, 1952, NRRL 2419).

Possible synonyms: *A. cyaneofuscatus* Gause *et al.* (Gause *et al.*, 1957).

A. levoris Krassilnikov (Krassilnikov, 1958).

Not *S. violaceoruber*.

Morphology: Sporophores of most strains short, arranged in small tufts, wavy; no spirals. Spores spherical to ellipsoidal; surface smooth.

Agar media: Substrate growth on most media colorless or atypical yellowish-brownish; sometimes pinkish-red, especially in the lower part of the slants. Aerial mycelium colored grayish-yellow, often with a greenish or pinkish shade. Soluble pigment on most media either absent or yellowish-brown. Blue pigment is produced by some strains on glucose-calcium malate-NH_4NO_3 agar, mannitol-calcium malate-peptone agar, or glucose-peptone agar.

Potato: Growth abundant, lichenoid. Aerial mycelium powdery, white to yellow. Characteristic formation of greenish-blue to

sky-blue soluble pigment by several strains; it may later become deep blue or blue-violet. Addition of glycerol delays pigment formation.

Gelatin: Good growth. Rapid liquefaction. No soluble pigment.

Milk: No coagulation, rapid peptonization, complete within 15 days at 22–27°C; coagulation within 3 to 5 days, followed by peptonization at 36°C.

Starch hydrolysis: Strong.

Nitrate reduction: Positive; none reported for *S. canescus* by Hickey *et al.* (1952).

Carbon sources: Utilizes L-xylose, L-arabinose, D-fructose, D-galactose, D-mannitol, salicin; does not utilize L-rhamnose or raffinose; most strains do not utilize sucrose.

Hemolysis of blood: Rapid at 37°C.

Production of H_2S: Negative.

Antagonistic properties: Active upon several fungi and yeasts; all strains as far as tested produce polyene antibiotics. *S. griseus* (Krainsky) Waksman and Curtis (1916) probably belongs to this species, since it is now known to produce an antifungal agent of the polyene type.

Ecology: *S. coelicolor* is widely distributed in nature. In a search for polyene-producing organisms, Pledger and Lechevalier (1955–1956) found 26 strains among 93 isolates which produced polyenes and which can be regarded as belonging to this species. Among the 382 subgroups of Kutzner (1956), the *S. coelicolor* subgroup was the one which comprised most strains. Heymer (1957) found this organism strikingly often on the skin and in the tonsils of men. The first culture of this species isolated by Müller (1908) and the ascosin-producing organism (*S. canescus*) were found as chance contaminants; this indicates the wide distribution of the organism in air. The relationship of blue pigment-forming bacteria, designated as *Actinobacillus* and *Actinococcus*, to this organism was discussed by Beijerinck (1913a).

Numerous cultures isolated by different investigators and described as *S. coelicolor* belong to *S. violaceoruber*. Others, however, such as *A. tricolor* Wollenweber, are related to *S. coelicolor*.

Type culture: A strain of this organism was deposited by R. Müller in the CBS.

59. *Streptomyces collinus* Lindenbein, 1952

Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Sporophores form spirals. Spores oval.

Glycerol nitrate agar: Growth yellow-brown to red-brown. Aerial mycelium chalk-white. Soluble pigment yellow-brown, later becoming reddish-brown.

Glucose-asparagine agar: Growth yellow-brown to purple-red. Aerial mycelium chalk-white, later ash-gray. Soluble pigment carmine-red, later brown-red.

Glycerol malate agar: Growth yellow-brown to red-brown. Aerial mycelium velvety, chalk-white. Soluble pigment yellow-brown.

Nutrient agar: Growth dark brown. Aerial mycelium powdery, gray-white. Soluble pigment dark brown. Melanin-positive.

Glucose-peptone agar: Growth yellow-brown and red. Aerial mycelium velvety, white. Soluble pigment chestnut-brown.

Starch media: Growth reddish to orange. Aerial mycelium white. Hydrolysis medium.

Potato: Growth good. Aerial mycelium white. No soluble pigment.

Gelatin: Growth dark brown. No aerial mycelium. Soluble pigment dark brown. Liquefaction rapid.

Milk: Growth good; dark brown reverse. Aerial mycelium white, later ash-gray. Soluble pigment dark brown. No peptonization.

Cellulose: Growth good, colorless.

Antagonistic properties: Produces an antibiotically active pigment.

Habitat: Soil.

Remarks: Closely related to *S. erythrochromogenes*. Gause *et al.* (1957) described a

similar form under the name of *A. albovinaceus*.

60. *Streptomyces coroniformis* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight, some long and some short. Spores oval, 0.8 by 0.6 μ.

Sucrose nitrate agar: Growth in form of discrete colonies partially coalescing, gray to greenish. Aerial mycelium white, covering edges of growth.

Nutrient potato agar: Growth wrinkled, grayish. No aerial mycelium.

Potato: Growth raised, grayish. Aerial mycelium white. Plug pigmented brownish around and under growth.

Gelatin: Growth fair. Liquefaction slow if any.

Milk: A few colonies on surface. No coagulation; peptonization limited.

Starch: No hydrolysis or trace.

Nitrate: Limited reduction to nitrite.

Tyrosinase reaction: Negative.

Temperature: Growth fair at 37.5°C.

Habitat: Potato scab.

61. *Streptomyces craterifer* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Straight sporophores form terminal branches, dichotomously forked. Spores rectangular, 1.3 to 0.9 by 1.0 to 0.8 μ.

Sucrose nitrate agar: Growth lichenoid, abundant, colorless; aerial mycelium mouse-gray. Numerous guttation drops, which leave blackish craters on surface of growth.

Nutrient agar: Growth colorless; aerial mycelium scant, white. No soluble pigment.

Starch agar: Growth spreading, thin, colorless; no aerial mycelium. Starch hydrolyzed.

Potato: Growth cream-colored; aerial mycelium white to mouse-gray. Color of plug unchanged.

Gelatin: Surface growth wrinkled; aerial mycelium white. Rapid liquefaction. No soluble pigment.

Milk: Surface growth cream-colored. No coagulation, rapid peptonization.

Tyrosinase reaction: Negative.

Nitrate reduction: Positive.

Temperature: Only slight growth at 37.5 C.

Habitat: Raised, smooth scab.

Type culture: IMRU 3373.

62. *Streptomyces cyaneus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 14, 1941).

Morphology: Sporophores produce open spirals (sinistrorse), with 2 to 3 turns in each. Spores oval, seldom spherical, 0.6 to 0.8 by 0.6 μ.

Agar media: Growth pigmented blue at both acid and alkaline reactions. The pigment does not dissolve into medium. Aerial mycelium well developed, downy, bluish-gray to blue-green in color.

Sucrose nitrate agar: Colonies at first smooth, becoming lumpy, leathery, compact, and covered with well developed blue-gray aerial mycelium.

Gelatin: Strong liquefaction. Melanin-positive.

Milk: Peptonization without prior coagulation.

Starch: Hydrolysis weak.

Cellulose: No growth.

Nitrate reduction: Negative.

Sucrose inversion: Negative.

Antagonistic properties: Weak.

Type culture: IMRU 3761.

63. *Streptomyces cyanoflavus* Funaki and Tsuchiya, 1958 (Funaki, M., Tsuchiya, F., Maeda, K., and Kamiya, T. J. Antibiotics (Japan) **11A:** 143–149, 1958).

Morphology: Sporophores straight, forming many branches, but no spirals.

Sucrose nitrate agar: Growth colorless to pale yellowish-brown. Aerial mycelium white to light greenish-gray. No soluble pigment.

Glucose-asparagine agar: Growth pale green to yellowish-brown. Aerial mycelium brownish-white to brownish-gray. Soluble pigment light blue to yellowish-brown.

Calcium malate agar: Growth light brownish to brown. Aerial mycelium white to gray. Soluble pigment greenish-blue to light brown.

Nutrient agar: Growth yellowish-brown to brown. Aerial mycelium grayish-white. No soluble pigment.

Yeast extract agar: Growth pale yellow to brown. Aerial mycelium light olive-gray. Soluble pigment brown.

Potato: Growth yellow to brown. Aerial mycelium brownish-gray. Soluble pigment dark brown.

Gelatin: Growth yellow. Soluble pigment yellow to brownish-yellow. Gelatin liquefied.

Milk: Produces a sedimented growth without any soluble pigment. Milk coagulated but not peptonized.

Carbon utilization: Utilizes various sugars and salts of organic acids, but not xylose, acetate, or citrate.

Antagonistic properties: Produces blue antibiotic cyanomycin, active against gram-positive and gram-negative bacteria; also produces aureothricin-like substances.

64. *Streptomyces cylindrosporus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 57, 1941).

Morphology: Sporophores straight, branched. Spores cylindrical or oblong, 1.0 to 1.7 by 0.7 μ.

Sucrose nitrate agar: Colonies velvety, dark brown or chocolate. Aerial mycelium white-gray to brown-gray. Soluble pigment brown.

Nutrient agar: Growth dark brown. Aerial mycelium white. Soluble pigment brown.

Glucose-asparagine agar: Growth brown. Aerial mycelium white-gray. No soluble pigment.

Gelatin: Weak liquefaction. Melanin-positive.

Milk: Coagulation limited, peptonization weak; color of milk brown to almost black.

Potato: Substrate growth brown. Aerial mycelium light gray. Soluble pigment brown.

Starch: Weak hydrolysis.

Cellulose: Limited, colorless growth. Aerial mycelium white.

Nitrate reduction: Positive.

Sucrose inversion: Negative.

Antagonistic properties: None.

Habitat: Soil.

Remarks: The description of the organism has been supplemented by Hoffmann (1958). It appears to be related to *S. vinaceus*, *S. purpurcochromogenes*, and *S. purpeofuscus*. Gause *et al.* (1957) described a related form as *A. umbrinus*.

Type culture: IMRU 3764.

65. *Streptomyces diastaticus* (Krainsky) Waksman and Henrici (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41:** 682, 1914).

Morphology: Sporophores form tight spirals. Spores oval or spherical, 1.0 to 1.2 by 1.1 to 1.5 μ (Figs. 37, 38).

Sucrose nitrate agar: Growth thin, gray. Aerial mycelium white, becoming drab gray.

Calcium malate agar: Colonies 2 to 4 mm, yellowish when old. Aerial mycelium gray, with white outer zone; white specks frequently produced in gray mycelium.

Glucose-asparagine agar: Growth yellowish, spreading. No aerial mycelium.

Nutrient agar: Growth cream-colored. Aerial mycelium white, then gray. Soluble brown pigment.

Potato: Growth white-gray. Aerial mycelium gray and white.

Gelatin: Liquefaction, with small, cream-colored flakes in liquefied part.

Milk: Brownish ring. Coagulation and slow peptonization.

Starch agar: Growth thin, colorless,

FIGURE 37. Sporophores of *S. diastaticus*, × 4,500, showing uniform density over whole surface (Courtesy of E. Baldacci, University of Milan, Italy).

spreading. Aerial mycelium gray. Ready hydrolysis.

Cellulose: Good growth.

Invertase: Negative.

Nitrate reduction: Weak.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Limited.

Habitat: Soil.

Remarks: This species was redescribed by Duché (1934) under the name *A. roseodiastaticus*. Baldacci *et al.* (1955) raised this species to the status of a "series." Several new species or varieties were created: *A. viridodiastaticus*, *A. diastaticus* var. *ardesicicus*, *A. diastaticus* var. *venezuelae*, *A. rubrocyanodiastaticus* var. *impiger* and var. *piger*.

Type culture: IMRU 3315.

66. *Streptomyces diastatochromogenes* (Krainsky, 1914; Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41**: 683, 1914).

Morphology: According to Waksman and Curtis (1916), sporophores are straight. According to Jensen (1930), sinistrorse spirals are produced. Spores spherical or oval, 1.2 μ.

Sucrose nitrate agar: Growth colorless, later yellowish-brown. Aerial mycelium abundant, white, later ash-gray. Soluble pigment yellowish to light brown.

Sucrose malate agar: According to Krainsky, growth colorless, with gray aerial mycelium. When glucose is added, center of aerial mycelium is colored yellowish, with gray margin.

Glucose-asparagine agar: Growth colorless, with gray aerial mycelium.

Nutrient agar: Aerial mycelium white to gray. Soluble pigment brownish to coffee-brown. Melanin-positive.

Potato: Growth light gray, later grayish-black. Aerial mycelium white to gray. Soluble pigment black.

Gelatin: Growth cream-colored to yellowish-brown. Aerial mycelium scant white. Sol-

FIGURE 38. Sporophores of *S. diastaticus*, × 15,000 (Courtesy of E. Baldacci, University of Milan, Italy).

uble pigment brown. Liquefaction fairly rapid.

Starch: Hydrolysis weak.

Cellulose: No growth.

Nitrate: Reduction to nitrite strong.

Tyrosinase reaction: Positive.

Temperature: Optimum 35°C.

Antagonistic properties: Very strong.

Habitat: Very common in soil.

Remarks: Krassilnikov (1949) considered this species as a variety of *A. chromogenes*.

Type culture: ATCC 12,309.

67. *Streptomyces echinatus* Corbaz *et al.*, 1957 (Corbaz, R., Ettlinger, L., Gäsmann, E., Keller-Schierlein, W., Kradolfer, F., Neipp, L., Prelog, V., Reusser, P., and

Zähner, H. Helv. Chim. Acta **40:** 199–204, 1957).

Morphology: Sporophores produce verticils on sterile aerial hyphae, with open, irregular spirals. Spores elliptical to oval; surface of spores covered with long, thin spines (Pl. I e).

Glycerol nitrate agar: Growth greenish-yellow to citron-yellow to light green. Aerial mycelium white, changing to yellow, to ash-gray. Soluble pigment greenish-yellow to grass-green.

Glycerol malate agar: Growth pale yellow, turning greenish-yellow. Aerial mycelium white to pale yellow. No soluble pigment.

Glucose-asparagine agar: Growth golden yellow to greenish-yellow to greenish-gray. Aerial mycelium ash-gray to reddish-violet. No soluble pigment.

Nutrient agar: Growth light yellow. No aerial mycelium. No soluble pigment.

Glucose-peptone agar: Growth yellow. Aerial mycelium white in center, brownish on periphery, changing to ash-gray. Soluble pigment golden yellow.

Starch agar: Growth yellow. Aerial mycelium ash-gray. No hydrolysis, or at most, traces.

Gelatin: Substrate growth dark brown. Aerial mycelium greenish-gray. Soluble pigment dark brown. No liquefaction after 31 days. Melanin-positive.

Potato: Growth greenish to raven-black. Aerial mycelium limited, white-gray to bluish-gray. Soluble pigment brownish to pitch-black.

Milk: Good coagulation and peptonization.

Tyrosinase reaction: Positive.

Carbon utilization: Xylose, lactose, raffinose, acetate, and succinate—positive. Sucrose, inulin, dulcitol, salicin, and sodium citrate—negative.

Temperature: Develops well at 18–40°C.

Antagonistic properties: Produces echinomycin.

Remarks: Closely related to *S. griseoflavus* and *S. flavcolus.*

68. *Streptomyces elasticus* (Söhngen and Fol, 1914) Waksman (Söhngen, N. L. and Fol, J. G. Centr. Bakteriol. Parasitenk. Abt. II, **40:** 87–98, 1914).

Morphology: Mycelium typical, branched. Short, motile rods observed in young cultures. Spores white, round, diameter about 1 μ, double that of the mycelium.

Agar media: Growth yellowish-white. Aerial mycelium snow-white.

Gelatin: Growth yellow-brown.

Carbon utilization: Glucose, glycerol, ethyl alcohol, mannitol, organic acids, calcium salts readily assimilated.

Sucrose inversion: Positive.

Urea: Produces urease.

Paraffin: Utilized.

Rubber: Utilized readily.

Temperature: Optimum 28°C, maximum 33°C, destroyed at 65°C in 5 minutes.

Habitat: Soil.

69. *Streptomyces endus* Gottlieb and Carter, 1956 (Gottlieb, D. and Carter, H. E. U. S. Patent 2, 746, 902, May 22, 1956).

Morphology: Sporophores formed along entire length of mycelium, at right angles to it. Compact spirals produced, often with 10 loops. Young hyphae 0.7 to 1.0 μ in diameter; old hyphae 1.25 to 1.50 and even 2.0 μ.

Sucrose nitrate agar: Substrate growth has color of medium, later turning dark. Aerial mycelium white, changing to light gray, then to dark gray. No soluble pigment.

Gelatin: Slow and only slight liquefaction. No soluble pigment. Melanin-negative.

Starch: Hydrolysis rapid.

Potato: Growth good. Aerial mycelium light gray. No soluble pigment.

Milk: Coagulation; no visible peptonization.

Carbon utilization: Utilizes starch, mannose, dextrin, glucose, arabinose, maltose,

and levulose. Poor growth with galactose, lactose, citric acid, malic acid, succinic acid, and cellulose. Does not utilize sucrose, sorbitol, dulcitol, inositol, or paraffin.

Antagonistic properties: Produces an antibiotic, endomycin, active largely upon fungi.

Remarks: Tresner and Backus (1956) consider this organism as a variant of the *S. hygroscopicus* group.

70. *Streptomyces erythraeus* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 99, 1916; Waksman, S. A. Soil Sci. **8:** 112, 1919).

Morphology: Fine, monopodially branched aerial mycelium; numerous sporophores with open and closed spirals. Spores spherical to oval, 0.7 to 0.8 μ, smooth (Pl. II m).

Sucrose nitrate agar: Growth yellowish, later becoming red. Pigment insoluble in medium. Aerial mycelium thick, white to pale rose.

Glucose-asparagine agar: Growth abundant, spreading, cream-colored, later turning brown chiefly on surface; center raised, lobate margin.

Nutrient agar: Substrate growth cream-colored. No soluble pigment.

Potato: Growth wrinkled, cream-colored, becoming yellowish to red to purplish. Melanin-negative.

Gelatin: Growth abundant, dense, gray with pinkish tinge, chiefly on surface of slowly liquefied portion. No soluble pigment.

Milk: Surface zone yellowish. Limited coagulation and positive peptonization.

Starch media: Growth cream-colored with faint greenish tinge. Hydrolysis.

Cellulose: Growth brick-red.

Invertase: None.

Nitrate: Reduction to nitrite only with starch.

Production of H_2S: Negative.

Temperature: Optimum 25°C.

Antagonistic properties: Marked. Produces erythromycin A and B.

Habitat: Soil.

Remarks: According to Ettlinger *et al.* (1958) *S. rimosus* and *S. roseochromogenes* belong to this group. Krassilnikov (1949) considers this organism as a variety of *A. ruber*. A closely related, melanin-positive culture has been described as a new species, *S. bottropensis* (Brit. Pat. 762, 736, Nov. 19, 1953).

Type culture: IMRU 3737; ATCC 11,635.

71. *Streptomyces erythrochromogenes* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41:** 679–682, 1914).

Different strains of this organism have been studied by Krainsky (1914), Waksman and Curtis (1916), Jensen (1930), and Okami and Suzuki (1958).

Morphology: Sporophores flexible, curved; spiral formation abundant according to Jensen and Okami and Suzuki. Waksman and Curtis reported no spirals. Spores oval.

Sucrose nitrate agar: Growth at first cream-colored, later turning red to violet to purple. Aerial mycelium white to light gray. Soluble pigment red to red-violet according to Jensen.

Calcium malate agar: Growth red to violet. Aerial mycelium grayish, with white margin.

Glucose-asparagine agar: Aerial mycelium gray to white. Soluble pigment red.

Nutrient agar: Growth yellowish-gray to light brown. Aerial mycelium white to light gray. Soluble pigment brown to deep brown.

Starch agar: Aerial mycelium gray. Soluble pigment rose-colored. Diastatic action weak.

Potato: Growth yellowish-gray, later almost black. Aerial mycelium gray. Soluble pigment black. Melanin-positive.

Gelatin: Growth yellowish to light purple. Liquefaction very slow. Soluble pigment brown.

Cellulose: Growth slow or none.

Invertase: Negative.

Nitrate: Reduction slight.

Pigment: Soluble in water, not in organic solvents.

Temperature: Optimum 30°C.

Antagonistic properties: Produces sarkomycin (Okami and Suzuki).

Habitat: Not very common in soil.

72. *Streptomyces eurocidicus* Okami *et al.*, 1954 (Okami, Y., Utahara, R., Nakamura, S., and Umezawa, H. J. Antibiotics (Japan) **7A**: 101–102, 1954).

Morphology: Aerial mycelium short, branched. Sporophores straight, without spirals; sometimes atypical verticils are produced.

Glycerol nitrate agar: Growth colorless to yellowish-brown. Aerial mycelium scant, thin, white. No soluble pigment.

Glucose-asparagine agar: Growth colorless to yellowish-brown. Aerial mycelium white with yellowish tinge. Soluble pigment absent or slightly brown.

Nutrient agar: Growth yellowish-brown to black. Soluble pigment brown. Melanin-positive (?).

Starch: Good hydrolysis.

Potato: Growth wrinkled, brownish-yellow. Aerial mycelium absent or thin white. No soluble pigment.

Gelatin: Growth yellowish-brown. Soluble pigment brown. No liquefaction.

Milk: Surface ring yellowish-brown.

Nitrate: No reduction.

Tyrosinase: Doubtful.

Antagonistic properties: Produces an antifungal substance, eurocidin, and antibacterial substances tertiomycin and azomycin.

73. *Streptomyces eurythermus* Corbaz *et al.*, 1955 (Corbaz, R., Ettlinger, L., Gäumann, E., Keller-Schierlein, W., Neipp, L., Prelog, V., Reusser, P., and Zähner, H. Helv. Chim. Acta **38**: 1202–1209, 1955).

Morphology: Substrate growth consists of long hyphae. Aerial mycelium abundant, gray. Sporophores broom-shaped. Spores egg-shaped to spherical, smooth, 0.8 to 1.0 by 0.6 to 0.7 μ.

Glycerol nitrate agar: Growth postulate light brown. Aerial mycelium sparse, white-gray, changing to ash-gray. Soluble pigment brown.

Nutrient agar: Growth brownish-yellow. Aerial mycelium ash-gray. Soluble pigment reddish-brown.

Glucose-asparagine agar: Growth thin, whitish yellow. Aerial mycelium white-gray to ash-gray. Soluble pigment chestnut-brown.

Starch agar: Growth golden yellow. Aerial mycelium velvety, at first snow-white, later gray. Soluble pigment light brown. Rapid hydrolysis.

Gelatin: Growth sparse. Soluble pigment dark brown. Rapid liquefaction.

Potato: Growth lichenoid, brownish-yellow. Aerial mycelium milky-white, becoming ash-gray. Soluble pigment brownish to pitch-black.

Milk: Brown surface ring. Aerial mycelium ash-gray. Soluble pigment dark brown. Coagulation and peptonization positive.

Carbon utilization: Xylose, arabinose, fructose, galactose, saccharose, maltose, lactose, mannitol, salicin well utilized. Rhamnose, inulin, sorbitol, dulcitol, meso-inositol not utilized. Some strains use acetate, citrate, and succinate.

Temperature: Poor growth at 18°C; very good growth at 30°C; good growth but no aerial mycelium at 58°C.

Antagonistic properties: Produces a basic antibiotic, angolamycin, related to erythromycin.

Habitat: Soil.

Remarks: Closely related to *S. antibioticus.*

74. *Streptomyces exfoliatus* (Waksman and Curtis, 1916) Waksman and Henrici, 1948

(Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 116, 1916; **8:** 121, 1919).

Morphology: Colony has tendency to crack and surface growth to exfoliate and peel off. Sporophores usually straight or slightly wavy; on some media there is a tendency to produce spirals. Spores oval, 1.0 to 1.5 by 1.2 to 1.8 μ.

Sucrose nitrate agar: Growth smooth, colorless, becoming brown to blue. Aerial mycelium white.

Malate-glycerol agar: Growth cream-colored. Aerial mycelium white. No soluble pigment.

Glucose-asparagine agar: Growth cream-colored, turning brown. Aerial mycelium white, appearing late.

Nutrient agar: Growth colorless. No aerial mycelium. Soluble pigment absent or brownish.

Potato: Growth wrinkled, gray, becoming brown. No aerial mycelium. No soluble pigment.

Gelatin: Growth cream-colored. Aerial mycelium white or absent. Liquefaction faint to fair. Melanin-negative.

Milk: Cream-colored ring. Soft coagulation and slow peptonization.

Starch media: Growth restricted, gray becoming brown. Aerial mycelium light buff-gray. Hydrolysis of starch medium, incomplete.

Invertase: Positive.

Cellulose: Growth good.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Positive.

Habitat: Soil.

Remarks: Krassilnikov (1949) considered this organism as a variety of one of the chromogenic groups.

Type culture: IMRU 3316.

75. *Streptomyces felleus* Lindenbein, 1952 (Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Sporophores long, straight, branching. Spores spherical, smooth.

Glycerol nitrate agar: Growth smooth, yellow-brown. Aerial mycelium velvety gray-white. Soluble pigment yellowish-brown.

Glucose-asparagine agar: Growth colorless to brownish-yellow. Aerial mycelium gray-white. Soluble pigment brownish.

Glycerol malate agar: Growth colorless to yellowish. Aerial mycelium powdery, gray-white. Soluble pigment yellowish-brown.

Nutrient agar: Growth colorless, brownish-yellow reverse. No aerial mycelium. Soluble pigment light brownish-yellow. Melanin-negative.

Glucose-peptone agar: Growth yellowish-brown. Aerial mycelium gray-white. Soluble pigment light brown.

Starch media: Growth lichenoid, colorless. Aerial mycelium white. No soluble pigment. Hydrolysis strong.

Potato: Growth brownish-yellow. No aerial mycelium. Soluble pigment absent or pinkish.

Gelatin: Growth colorless. No aerial mycelium. No soluble pigment. No liquefaction by one strain, positive by another.

Milk: Growth brownish to orange. Aerial mycelium gray-white. Peptonization medium.

Cellulose: No or weak growth.

Production of H_2S: Negative.

Odor: Typical earthy.

Taste: Gall-bitter.

Antagonistic properties: Produces antibiotic picromycin.

Remarks: Related to *S. fimicarius*. Ettlinger *et al.* (1958) considered this organism as belonging to *S. olivaceus*.

Type culture: IMRU 3659.

76. *Streptomyces fervens* DeBoer *et al.*, 1959 (DeBoer, C., Dietz, A., Evans, J. S., and Michaels, R. M. Antibiotics Ann. 1959–1960, pp. 220–226).

Morphology: Sporophores monoverticillate or biverticillate. Pigment granules present in mycelium.

Sucrose nitrate agar: Growth faint–pink. Aerial mycelium pink. No soluble pigment.

Calcium malate agar: Growth pink. Aerial mycelium trace, pink. No soluble pigment.

Glucose-asparagine agar: Growth pink. Aerial mycelium pink. Soluble pigment pale yellow.

Starch nutrient agar: Growth red-pink. Aerial mycelium pink. Soluble pigment tan.

Casein digest-beef extract agar: Growth red. Aerial mycelium pink.

Gelatin: Liquefaction medium. Soluble pigment brown.

Milk: Growth brown to cream-pink. Aerial mycelium trace pink. No coagulation. No peptonization.

Production of H_2S: Positive.

Starch: Hydrolyzed.

Carbon utilization: Utilizes various carbohydrates, glycerol, inositol, starch, certain organic acids (acetate, citrate, succinate); does not utilize *d*-xylose, rhamnose, lactose, *l*-arabinose, formic, oxalic, and tartaric acids.

Antagonistic properties: Produces antibiotic fervenulin, active against various microbes and tumors.

Habitat: Soil in California.

77. *Streptomyces filamentosus* Okami *et al.*, 1953 (Okami, Y., Okuda, T., Takeuchi, T., Nitta, K., and Umezawa, H. J. Antibiotics (Japan) **6A:** 153–157, 1953).

Morphology: Sporophores straight, long, without spirals. Spores oval to elliptical.

Sucrose nitrate agar: Growth colorless. Aerial mycelium abundant, cottony, white. No soluble pigment.

Glucose-asparagine agar: Growth colorless. Aerial mycelium abundant, white with pinkish-orange tinge or brownish to almost salmon-pink tinge. No soluble pigment.

Nutrient agar: Growth colorless. Aerial mycelium thin, white. No soluble pigment.

Starch agar: Growth same as on synthetic agar. Aerial mycelium white or white with light brownish-salmon-pink tinge. Hydrolysis.

Gelatin: Growth yellowish. Aerial mycelium in form of white patches. No soluble pigment. Medium liquefaction.

Potato plug: Growth cream-colored, wrinkled. No aerial mycelium. No soluble pigment.

Milk: Growth yellowish, surface ring. Aerial mycelium white, scant. No soluble pigment. Coagulation and peptonization.

Blood agar: Growth brownish, wrinkled. No aerial mycelium. Hemolysis none or weak.

Antagonistic properties: Produces caryomycin, an antitumor substance.

78. *Streptomyces filipinensis* Ammann *et al.*, 1955 (Ammann, A., Gottlieb, D., Brock, T. D., Carter, H. E., and Whitfield, G. B. Phytopathology **45:** 559–563, 1955).

Morphology: Sporophores form spirals that vary from open to tightly closed. Spores round to oval.

Sucrose nitrate agar: Growth excellent, light yellow. Aerial mycelium cottony, white, turning gray. Soluble pigment slightly yellow. Colorless drops of exudate on mycelium.

Starch-nitrate agar: Growth excellent. Aerial mycelium velvety, white, turning gray. No soluble pigment. Hydrolysis weak.

Glycerol-asparagine agar: Growth excellent. Aerial mycelium white, turning gray. Soluble pigment slightly yellow.

Nutrient agar: Growth very poor, light buff. No aerial mycelium. Soluble pigment brown.

Gelatin: Slow but definite liquefaction, stratiform type. Soluble pigment brown.

Potato: Growth good. No aerial mycelium. Soluble pigment purple to black.

Nitrate reduction: Little, if any.

Production of H_2S: Positive.

Carbon utilization: Utilizes xylose, arabi-

nose, fructose, galactose, sucrose, maltose, lactose, raffinose, inulin, mannitol, inositol, sodium acetate, sodium citrate, sodium succinate, dextrose, mannose, starch, dextrin, and glycerol. Does not utilize rhamnose, sorbitol, dulcitol, salicin, phenol, *m*-cresol, sodium formate, sodium oxalate, sodium tartrate, or sodium salicylate.

Antagonistic properties: Produces antifungal agent filipin, of the polyene type.

Habitat: Philippine soil.

Type culture: IMRU 3781.

79. *Streptomyces fimbriatus* (Millard and Burr, 1926) Waksman and Henrici, 1948 (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores form spirals with three or more turns. Spores cylindrical to oval, 1.2 to 0.9 by 0.9 μ.

Sucrose nitrate agar: Growth gray. Aerial mycelium abundant, white to gray. Soluble pigment cream-colored.

Glucose-asparagine agar: Growth very good. Aerial mycelium white to mouse-gray.

Nutrient potato agar: Colonies gray to blackish, flat, raised in center. Aerial mycelium a few specks of white. Soluble pigment golden brown.

Potato: Growth mouse-gray. Aerial mycelium on dried portions of growth scant, white to mouse-gray. Pigment around growth black.

Gelatin: Growth good. Aerial mycelium white. Liquefaction slow. Soluble pigment reddish.

Milk: Growth good. No coagulation and no hydrolysis.

Starch: Positive hydrolysis.

Tyrosinase reaction: Strongly positive.

Nitrate reduction: Positive.

Habitat: Common scab of potatoes.

80. *Streptomyces fimicarius* (Duché, 1934) Waksman and Henrici, 1948 (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Morphology: Sporophores long, tuft-forming; no spirals. Spores cylindrical (Hoffmann, 1958).

Sucrose nitrate agar: Growth at first colorless, later yellowish to red-brown; reverse orange-colored. Aerial mycelium light gray with yellowish tone. Soluble pigment faint yellowish.

Glucose-asparagine agar: Growth cream-colored with whitish aerial mycelium; reverse cream-colored to slight ocher.

Nutrient agar: Growth limited, cream-colored with white aerial mycelium; reverse yellowish.

Potato: Growth cream-colored to yellowish to dark brown. Aerial mycelium gray. Soluble pigment reddish-brown.

Gelatin: Punctiform colonies with whitish aerial mycelium. Soluble pigment reddish. Liquefaction medium. Melanin-negative.

Milk: Growth colorless, becoming covered with whitish aerial mycelium. Slow peptonization. Pigment rose, changing to brownish-red.

Starch: Hydrolyzed.

Coagulated serum: Growth cream-colored. Aerial mycelium whitish. Liquefaction rapid.

Cellulose: No growth.

Tyrosine medium: Growth white, with yellowish reverse. Soluble pigment yellowish.

Production of H_2S: Negative.

Antagonistic properties: Positive.

Remarks: Krassilnikov (1949) considers this organism as a variety of *A. chromogenes*.

81. *Streptomyces flavcolus* (Waksman) Waksman and Henrici, 1948 (Waksman, S. A. No. 168. Soil Sci. **8:** 134, 1919).

Morphology: Sporophores monopodially branched. Short, closed and open spirals produced on all media. Spores oval to elliptical, 0.8 by 1.2 μ, covered with long, fine hair.

Sucrose nitrate agar: Growth light sulfur-yellow, turning cadmium-yellow. Aerial mycelium white to ash-gray. Soluble pigment yellow.

Malate-glycerol agar: Growth colorless to cream-colored. Aerial mycelium mouse-gray.

Glucose-asparagine agar: Aerial mycelium pale gray. Soluble pigment yellowish-green.

Nutrient agar: Growth colorless, glistening, wrinkled. Aerial mycelium white. Soluble pigment absent or yellow.

Potato: Growth abundant, wrinkled, cream-colored to yellow. Aerial mycelium white to pinkish. Soluble pigment absent or faint brown.

Gelatin: Growth abundant, yellowish, spreading. Aerial mycelium white. Liquefaction rapid. Soluble pigment yellowish-brown, not melanoid.

Milk: Ring sulfur-yellow. Rapid coagulation and strong peptonization.

Starch media: Growth colorless. Aerial mycelium light gray. Hydrolysis.

Cellulose: Growth scant.

Invertase: Negative.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Antagonistic properties: Some strains produce actinomycin.

Habitat: Soil.

Remarks: Several varieties of this organism have been described. It is sufficient to mention a culture described by Krassilnikov as *A. rectus*, which appears to be a variety of *S. flaveolus*. Krassilnikov also believed that *A. krainskii* Duché belongs to this group.

Type culture: IMRU 3319.

82. *Streptomyces flavochromogenes* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41**: 685, 1914).

Morphology: Spores oval, 1.7 μ.

Glucose-asparagine agar: Growth yellow. Aerial mycelium gray. Soluble pigment brown.

Calcium malate agar: Growth yellow. Aerial mycelium produced late, white to gray.

Nutrient agar: Aerial mycelium formed late, at first white, later gray. Soluble pigment brown.

Starch agar: Growth yellow. Aerial mycelium white. Weakly diastatic.

Potato: Growth yellow. Aerial mycelium white. Soluble pigment black.

Gelatin: Colonies yellowish. Slight liquefaction. Soluble pigment brown.

Cellulose: Growth slow.

Nitrate reduction: Strong.

Tyrosinase: Positive.

Temperature: Optimum 35 C.

Habitat: Garden soil.

Remarks: Krainsky considered this culture as identical to *A. chromogenes* Gasperini.

Type culture: IMRU 3671.

83. *Streptomyces flavogriseus* (Duché, 1934) Waksman (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Morphology: Sporophores long, straight, with a few curling tips. Spores spherical.

Sucrose nitrate agar: Growth limited, yellowish, reverse turning black. Aerial mycelium thin, gray to mouse-gray.

Nutrient agar: Growth thin, cream-colored. Aerial mycelium thin, white. No soluble pigment. Melanin-negative.

Glucose-peptone agar: Growth yellow; reverse tending to turn dark. Aerial mycelium abundant, mouse-gray to drab. No soluble pigment.

Starch agar: Growth very limited, similar to that on sucrose nitrate agar. Hydrolysis.

Potato: Growth abundant, lichenoid. Aerial mycelium abundant, mouse-gray to drab with white edge. No soluble pigment.

Gelatin: Growth flocculent, through medium. Liquefaction slow. No soluble pigment.

Milk: Cream-colored ring. No aerial mycelium. Peptonization very rapid.

Remarks: According to Ettlinger *et al.* (1958) this organism belongs to the *S. fradiae* group.

Type culture: IMRU 3322.

84. *Streptomyces flavoreticuli* Funaki *et al.*, 1958 (Funaki, M., Tsuchiya, F., Maeda, K., and Kamiya, T. J. Antibiotics (Japan) **IIA:** 138–142, 1958).

Morphology: Aerial mycelium is long with many short branches; numerous small verticils are produced depending on the nature of the medium, especially on starch-ammonium agar.

Sucrose nitrate agar: Growth colorless to pale yellow. Aerial mycelium white, cottony. Soluble pigment faint yellow.

Glucose-asparagine agar: Growth pale yellow to yellowish-brown. Aerial mycelium yellowish-white to olive-gray. Soluble pigment pale yellow.

Calcium malate agar: Growth pale yellow. Aerial mycelium pale yellow. No soluble pigment.

Nutrient agar: Growth yellow to brown. Aerial mycelium pale yellow to yellowish-gray. Soluble pigment brown.

Potato: Growth yellow, folded. Aerial mycelium yellowish white to olive-gray or olive-yellow. Soluble pigment dark brown.

Milk: Colorless pellicle. Aerial mycelium white. Soluble pigment brown. Milk coagulated, then peptonized.

Gelatin: Growth yellowish-brown. Aerial mycelium white to light gray. Soluble pigment brown. Gelatin liquefied.

Starch: Not hydrolyzed.

Antagonistic properties: Produces antibiotic virocidin, which possesses antiviral and antibacterial properties.

Remarks: Similar to *S. reticuli* and to *S. flavus*, differing from the first by the formation of yellow growth and yellow soluble pigment and from the second by the formation of verticils.

85. *Streptomyces flavovirens* (Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. Soil Sci. **8:** 117, 1919).

Morphology: Sporophores coarse, straight, and short, relatively unbranched; large masses of minute tufts; open spirals may be produced in certain substrates. Spores spherical, oval to rod-shaped, 0.75 to 1.0 by 1.0 to 1.5 μ.

Sucrose nitrate agar: Growth yellowish with greenish tinge. Aerial mycelium gray. Soluble pigment greenish-yellow.

Glucose-asparagine agar: Growth restricted, developing only to a very small extent into the medium, yellow, turning black. Soluble pigment golden yellow to greenish-yellow.

Nutrient agar: Growth yellowish; reverse dark in center with yellowish zone and outer white zone.

Potato: Growth sulfur-yellow, wrinkled.

Gelatin: Surface pellicle yellowish-green. Good liquefaction. Melanin-negative.

Milk: Cream-colored to brownish ring; coagulation and peptonization.

Starch agar: Growth greenish-yellow, spreading, developing deep into the medium. Good hydrolysis.

Invertase: Negative.

Nitrate reduction: Limited.

Production of H_2S: Negative.

Cellulose: No growth.

Antagonistic properties: Produces actinomycin.

Habitat: Soil.

Remarks: Certain forms belonging to this species, such as *A. griseostramineus* and *A. olivaceoviridis*, have been described by Gause *et al.* (1957). Ettlinger *et al.* (1958) considers this species as belonging to the *S. fradiae* group. Hirsch (1960) considers this organism as an oligonitrophilic form.

Type culture: IMRU 3320.

86. *Streptomyces flavus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41:** 685, 1914; Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 99, 1916; **3:** 71, 1919).

Not *A. flavus* Krainsky *emend.* Krassilnikov (1941).

Morphology: Sporophores are long, usu-

ally no spirals; some open spirals may be produced. Spores oval, 1.2 μ.

Sucrose nitrate agar: Growth yellow or sulfur-yellow. Aerial mycelium straw-yellow.

Glucose-asparagine agar: Growth sulfur-yellow, center shading to brown. Aerial mycelium white to gray.

Nutrient agar: Growth gray, spreading, folded. Aerial mycelium white, appears late.

Starch agar: Growth cream-colored with pink tinge. Hydrolysis marked.

Potato: Growth yellow. Aerial mycelium gray. Melanin-negative.

Gelatin: Growth in form of small, yellowish masses on surface. Rapid liquefaction. Melanin-negative.

Milk: Rapid coagulation and peptonization.

Sucrose inversion: Negative.

Nitrate: No reduction.

Cellulose: Growth poor.

Temperature: Optimum 25°C.

Antagonistic properties: Some strains produce actinomycin and certain other antibiotics.

Habitat: Soil.

Remarks: Represents a large group of species, as shown previously (Chapter 3). Above description is based largely upon that given by Krainsky. According to Ettlinger *et al.* (1958), this organism does not form any spirals (as found also by Waksman and Curtis) and is related to *S. olivaceus*.

Type culture: IMRU 3321.

87. *Streptomyces flocculus* (Duché, 1934) Waksman and Henrici (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Sucrose nitrate agar: Growth cream-colored, later covered with white aerial mycelium. No soluble pigment.

Glucose-asparagine agar: Growth limited, cream-colored, only slightly raised above the surface of the medium; occasionally abundant growth produced with white aerial mycelium, colorless on reverse side.

Nutrient agar: Growth cream-colored, later covered with white aerial mycelium. No soluble pigment.

Potato: Growth punctiform. Aerial mycelium white. Soluble pigment faint, yellowish.

Gelatin: Growth limited. Liquefaction slow. Melanin-negative.

Milk: Growth rose-colored. Peptonization slow.

Coagulated serum: Growth cream-colored. Aerial mycelium fine, white. Liquefaction slow.

Tyrosine medium: Growth whitish. No soluble pigment.

Production of H_2S: Negative.

Remarks: Belongs to the *S. albus* series.

88. *Streptomyces fradiae* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 99–134, 1916; **8:** 90, 1919).

Morphology: Sporophores branched monopodially, straight or flexible, but no true spirals. On certain media, such as glycerol agar, spirals are formed. Ettlinger *et al.* (1958) found open spirals. Spores oval to rod-shaped, 0.5 by 0.7 to 1.25 μ, smooth (Fig. 39).

Sucrose nitrate agar: Growth smooth, spreading, colorless, or pale yellow-orange. Aerial mycelium thick, cottony, seashell-pink. No soluble pigment.

Malate-glycerol agar: Growth orange. Aerial mycelium seashell-pink.

Glucose-asparagine agar: Growth restricted, glossy, buff-colored, lichenoid margin. Aerial mycelium appears late, seashell-pink.

Nutrient agar: Growth restricted, yellowish, becoming orange-yellow to buff. No aerial mycelium. No soluble pigment.

Potato: Growth restricted, orange-colored. Aerial mycelium white to rose or pink. Soluble pigment absent or faint brown.

Gelatin: Growth dense, cream-colored to brownish. Aerial mycelium white. Gelatin liquefied. No soluble pigment.

FIGURE 39. Sporophores of *S. fradiae* (Prepared by H. Lechevalier of the Institute of Microbiology).

Starch media: Growth spreading, colorless. Aerial mycelium seashell-pink. Good diastatic action.

Milk: Cream-colored ring; coagulation and rapid peptonization.

Nitrate: Varied reduction.

Cellulose: No growth in solution, fair growth on plates.

Production of H_2S: Negative.

Invertase: None.

Antagonistic properties: Highly antagonistic. Produces an antibacterial agent, neomycin, and an antifungal agent, fradicin.

Habitat: Soil.

Remarks: A number of strains of this organism have been isolated from various soils (see, for example, *S. decaris* described as No. 3719, in Waksman *et al.*, 1958). Some vary in their pigmentation, rate of gelatin liquefaction, and antibiotic production; *A. longissimus* Krassilnikov is one such typical strain. Some strains are able to produce antiviral substances, as in the case of luridin, produced by a strain of *S. fradiae* designated as *A. luridus* by Krassilnikov *et al.* Gause *et al.* (1957) described a spiral-producing variety of *S. fradiae* under the name *spiralis*. Several other such strains have been isolated by Waksman and Lechevalier, Umezawa, and many others. Ettlinger *et al.* (1958) claimed that *S. rochei*, *S. filipinensis*, *S. coelicolor*, *S. flavogriseus*, *S. tyrosinaticus*, *S. violaceus*, and *S. violaceoruber* belong to this group; this claim cannot be accepted on the basis of evidence submitted in the descriptions of these organisms. The characteristics of the species are that it is nonchromogenic, strongly proteolytic, and produces the characteristic seashell-pink aerial mycelium on various synthetic media; on organic media, orange-colored growth is produced without any aerial mycelium.

Type culture: IMRU 3535.

89. *Streptomyces fragilis* Anderson *et al.*, 1956 (Anderson, L. E., Ehrlich, J., Sun, S. H., and Burkholder, P. R. Antibiotics & Chemotherapy **6:** 100, 1956).

Morphology: Aerial hyphae simple or branched, usually in small clusters; short, straight, or slightly curved, with bent or curved tips and occasional short spirals. Spores spherical to ovoid, 0.8 to 1.5 by 1.0 to 2.0 μ.

Calcium malate agar: Growth sparse. Aerial mycelium light brown.

Glycerol-asparagine agar: Growth sparse, colorless to light yellow. Aerial mycelium light yellow-pink.

Starch-ammonium sulfate agar: Growth yellow to yellow-orange to orange-brown. Aerial mycelium light yellow-pink to light brown.

Nutrient agar: Growth yellow to yellow-orange to brown. Aerial mycelium white to light yellow-pink. Melanin-negative.

Glucose-tryptone agar: Growth yellow to yellow-orange to brown. Aerial mycelium light yellow-pink, occasionally pink to gray-pink.

Gelatin: Liquefaction slow. No soluble pigment.

Litmus milk: Slow peptonization.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Carbon utilization: Utilizes L-arabinose, D-cellobiose, dextrin, D-galactose, glucose, D-maltose, starch, trehalose, and D-xylose.

Does not utilize aesculin, adonitol, cellulose, citrate, dulcitol, glycerol, *i*-inositol, inulin, D-lactose, D-levulose, D-mannitol, D-mannose, melezitose, melibiose, D-raffinose, L-rhamnose, salicin, D-sorbitol, succinate, or sucrose.

Antagonistic properties: Produces a substance, azaserine, that possesses certain anticancer properties.

Source: Argentine soil.

Remarks: Closely resembles *S. fradiae*. A detailed comparison between this and closely related organisms has been made by Anderson *et al.* (1956).

Type culture: IMRU 3732, NRRL 2424.

90. *Streptomyces fulvissimus* (Jensen, 1930) Waksman and Henrici, 1948 (Jensen, H. L. Soil Sci. **30:** 66, 1930).

Morphology: Sporophores short, straight, often trifurcated. Slightly wavy, but no true spirals. Spores oblong, smooth, 1.0 to 1.2 by 1.2 to 1.5 μ (Pl. I d).

Sucrose nitrate agar: Growth light golden, later deep orange to red-brown. Aerial mycelium scant, white, later grayish-brown. Soluble pigment bright golden to orange.

Glycerol-asparagine agar: Growth golden to dark brown. Aerial mycelium white to light cinnamon-brown. Soluble pigment golden to orange.

Nutrient agar: Growth wrinkled, deep red-brown. No aerial mycelium. Soluble pigment brownish-yellow. Melanin-negative.

Potato: Growth wrinkled, rust-brown. Aerial mycelium absent or white. Soluble pigment gray to faint lemon-yellow.

Gelatin: Growth yellowish-brown to red-brown. No aerial mycelium. No soluble pigment. Rapid liquefaction.

Starch-casein agar: Growth yellowish-brown. Aerial mycelium hydrolyzed, smooth, lead-gray. Soluble pigment dull yellow to orange starch.

Production of H_2S: Positive.

Antagonistic properties: Produces valinomycin.

Habitat: Very common in soil.

Remarks: The characteristic golden pigment is formed in nearly all media, but becomes most typical and attains its greatest brightness in synthetic agar media. It has indicator properties, turning red in strongly acid solutions. The species is easily recognized on agar plates by its bronze-colored colonies, surrounded by halos of bright yellow pigment.

This species was believed to be identical with the culture described by Millard and Burr (1926) as *A. flavus*. The last name is invalid, however, since the culture could be readily distinguished from the *S. flavus* of Krainsky (1914, *emend.* Waksman and Curtis, 1916) Waksman and Henrici.

Type culture: IMRU 3665.

91. *Streptomyces fumosus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 58, 1941).

Morphology: Sporophores straight. Spores cylindrical, later round, 1.5 to 2.0 by 0.7 μ.

Sucrose nitrate agar: Growth dark brown, pigment insoluble. Aerial mycelium well developed, cottony, dust-colored, occasionally gray-white.

Nutrient agar: Growth dark brown. Aerial mycelium white. Soluble pigment brown.

Potato: Aerial mycelium absent or only faint, dark gray. Melanin-negative.

Gelatin: Liquefaction medium.

Milk: No coagulation; slow liquefaction. Soluble pigment dark brown to almost black.

Starch: Good hydrolysis.

Cellulose: No growth.

Sucrose: Inversion weak.

Antagonistic properties: None

Habitat: Soil.

92. *Streptomyces fungicidicus* Okami *et al.*, 1954 (Okami, Y., Utahara, R., Nakamura, S., and Umezawa, H. J. Antibiotics (Japan) **7A:** 100–101, 1954).

Morphology: Aerial mycelium produces

numerous spirals on most synthetic media. Spores spherical to oval.

Glycerol nitrate agar: Growth colorless. Aerial mycelium white to grayish.

Glucose-asparagine agar: Growth colorless. Aerial mycelium white to grayish. Soluble pigment sometimes yellowish.

Calcium malate agar: Growth colorless to yellowish. Aerial mycelium white to grayish. Soluble pigment of some strains pink; later disappears.

Nutrient agar: Soluble pigment absent or slightly yellowish-brown. Melanin-negative.

Starch agar: Growth colorless to yellowish. Aerial mycelium white to grayish. Strong hydrolysis.

Potato: Growth yellowish to grayish. Aerial mycelium absent or white to grayish. A deep brown soluble pigment around the growth may be produced.

Milk: Growth colorless to cream-colored. Soluble pigment absent or slightly brown. Coagulation and peptonization weak.

Gelatin: Growth colorless to cream-colored. Soluble pigment absent or faint brown. Positive liquefaction.

Nitrate: No, or doubtful reduction.

Tyrosinase: Some strains positive.

Antagonistic properties: Produces a polyene-type antifungal substance, fungicidin.

Remarks: Two groups of this species were recognized; Group A produces nonspiral-forming sporophores; aerial mycelium white; violet pigment on potato. It is thus differentiated from Group G, described above.

93. *Streptomyces fuscus* (Söhngen and Fol, 1914) Waksman (Söhngen, N. L. and Fol, J. G. Centr. Bakteriol. Parasitenk. Abt. II, **40:** 89–98, 1914).

Morphology: According to Krassilnikov, the organism forms short straight sporophores arranged in fascicles or clusters in the form of brushes.

Agar media: Growth irregular, dry, colorless to stone-red. Aerial mycelium initially white, later becoming dark brown. Spores rose-colored. Some strains excrete a brown substance in protein media.

Carbohydrates: Slight decomposition; even glucose is assimilated with difficulty.

Carbon utilization: Best sources are calcium salts of various organic acids, ranging from malic and citric to stearate and palmitate. Formate not utilized.

Nitrogen utilization: Ammonium chloride and asparagine, nitrate, and peptone assimilated with difficulty.

Paraffins: Assimilated.

Rubber: Brown-red growth. Rubber decomposed.

Temperature: Optimum 33°C; maximum 37°C. Destroyed in 5 minutes at 65°C.

Habitat: Soil.

94. *Streptomyces galbus* Frommer, 1959 (Frommer, W. Arch. Mikrobiol. **32:** 195, 1959).

Morphology: Sporophores monopodially branched, ending in spirals with 3 to 8 turns. On some media, certain strains produce long, straight, slightly branched aerial hyphae, with short side branches.

Glycerol nitrate agar: Growth abundant; reverse yellow to yellow-green. Aerial mycelium cream-colored, mouse-gray, or green-gray. Soluble pigment golden yellow, later turning green-yellow.

Glucose-asparagine agar: Growth weak, crusty, reverse light yellow or green-yellow, later turning brown. Aerial mycelium thin, powdery, mouse-gray with white spots. Soluble pigment yellowish to yellow-green.

Calcium malate agar: Growth yellow to greenish-yellow. Aerial mycelium white to brownish-gray. Soluble pigment yellow to yellow-green.

Nutrient agar: Growth thin, brown. No aerial mycelium. Soluble pigment brown.

Starch-KNO_3 agar: Growth yellow. Aerial mycelium white to white-gray. Soluble pigment yellow. Slow hydrolysis of starch.

Potato: Growth heavy, yellow to reddish-brown. Aerial mycelium powdery, white,

mouse-gray to green-gray. Soluble pigment black.

Gelatin: Growth abundant. Aerial mycelium yellow. Soluble pigment dark brown. Slow liquefaction.

Milk: No coagulation; slow peptonization.

Cellulose: Growth good.

Antagonistic properties: Produces actinomycin.

Remarks: Related to *S. viridochromogenes*, *S. flavochromogenes*, and *S. viridoflavus*. It was also said to be related to *S. parvullus*. A variety of this species designated as *achromogenes*, not producing any melanin pigment, was also described.

95. *Streptomyces galilaeus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 356, 1958).

Morphology: Sporophores monopodially branched, with open, regular spirals. Spores smooth (Pl. II k).

Glycerol nitrate agar: Substrate growth at first light carmine, later carmine-red. Aerial mycelium white-gray. No soluble pigment.

Glucose-asparagine agar: Growth thin, white-yellow, later red. Aerial mycelium ash-gray. No soluble pigment.

Calcium malate agar: Growth thin, white-yellow, later red. Aerial mycelium ash-gray. No soluble pigment.

Starch agar: Growth carmine-red. Aerial mycelium white-gray. Limited hydrolysis.

Potato: Growth brownish-yellow. Aerial mycelium ash-gray. Soluble pigment limited, chestnut-brown. Melanin-positive.

Gelatin: Surface growth light red to light brown. Aerial mycelium sparse, grayish-white. Soluble pigment reddish-brown to dark brown. Liquefaction trace. Melanin-positive.

Milk: Pellicle thick, light brown. Aerial mycelium ash-gray. Limited coagulation, no peptonization.

Antagonistic properties: Positive.

Habitat: Soil.

96. *Streptomyces galtieri* (Goret and Joubert, 1951) Waksman (Goret, P. and Joubert, L. Ann. parasitol. humaine et comparée **26:** 118–127, 1951).

Morphology: Two types of colonies are produced on agar: one small, flat, regular, white; the other large, thick, irregular, yellowish. Sporophores form spirals. Spores oval, 0.8 to 1.5 by 0.8 μ.

Sucrose nitrate agar: Growth limited. Aerial mycelium powdery, white. No soluble pigment.

Nutrient agar: Growth poor, thin, yellowish. Aerial mycelium powdery, white. Soluble pigment brown.

Peptone agar: Growth limited, cream-colored. Aerial mycelium powdery, white. Soluble pigment very slight, brown-reddish.

Starch agar: Growth thin. Aerial mycelium powdery, white. No soluble pigment.

Potato: Punctiform colonies growing together as thick crust, orange-reddish in color. Aerial mycelium limited, white, appearing very slowly. No soluble pigment.

Gelatin: Growth poor, flaky, white. Liquefaction limited.

Milk: Growth slow. Aerial mycelium white. At 25°C no coagulation; at 37°C coagulation after 20 days; no peptonization.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Source: Dog septicemia (thoracic, abdominal, and brain lesions).

Remarks: Said to be pathogenic for guinea pig and rabbit. Culture grown in the laboratory not pathogenic for dogs.

97. *Streptomyces gardneri* (Waksman and Henrici) nov. comb. (Gardner, A. D. and Chain, E. Brit. J. Exptl. Pathol. **23:** 123, 1942; Waksman, S. A., Horning, E. S. Welsch, M., and Woodruff, H. B. Soil Sci. **54:** 289, 1942).

Morphology: When grown on oatmeal agar, aerial mycelium thin, largely at edge of growth, consisting of short, straight to

wavy sporophores, often produced in clusters; no spirals.

Glucose-asparagine agar: Growth brownish, lichenoid, with wide cream-colored edge; reverse yellowish. Aerial mycelium white to grayish, gradually covering surface. No soluble pigment.

Nutrient agar: Growth cream-colored, elevated, lichenoid, doughy consistency. No aerial mycelium. Soluble pigment faint brownish.

Potato: Growth barnacle-like, reddish-brown. No aerial mycelium. Pigment around growth grayish-brown.

Gelatin: Surface ring cream-colored. Liquefaction medium. Soluble pigment deep brown, gradually diffusing through liquefied portion.

Tryptone broth: Growth occurs in form of small pellets at the base of the flask; later, a thin surface pellicle is produced. Soluble pigment produced slowly, black.

Temperature: Good growth at 25°C; slow growth at 37°C.

Antagonistic properties: Produces on synthetic and organic media an antibiotic, proactinomycin, active against bacteria.

Source: Isolated as an air contaminant.

Remarks: This species was first classified as a *Nocardia* (Bergey's Manual, 7th ed.). Recent evidence, comprising both cultural and chemical properties (Cummins and Harris, 1958), suggests its transfer to the genus *Streptomyces*. It is closely related to *S. aureofaciens*.

Type culture: IMRU 3834.

98. *Streptomyces garyphalus* Harris *et al.*, 1955 (Harris, D. A., Ruger, M., Reagan, M. A., Wolf, F. J., Peck, R. L., Wallick, H., and Woodruff, H. B. Antibiotics & Chemotherapy **5:** 183–190, 1955).

Morphology: Sporophores straight, without spirals. Spores rod-shaped, 0.8 to 1.1 by 1.7 to 1.9 μ.

Sucrose nitrate agar: Growth colorless. Aerial mycelium grayish-white. No soluble pigment.

Glucose-asparagine agar: Growth colorless. Aerial mycelium white. No soluble pigment.

Modified glucose-asparagine agar: Growth powdery, pinkish-white, reverse buff. Aerial mycelium seashell-pink. No soluble pigment.

Calcium malate agar: Growth colorless No aerial mycelium.

Nutrient agar: Growth colorless. Aerial mycelium grayish-white. Soluble pigment faint brown.

Yeast extract-glucose agar: Growth excellent. Aerial mycelium grayish-white, becoming pinkish-gray and finally seashell-pink. Soluble pigment faint brown.

Starch-tryptone agar: Growth good. Aerial mycelium gray. Soluble pigment dark brown.

Peptone-glucose agar: Growth cream-colored. Aerial mycelium grayish-white, becoming pink. Soluble pigment faint brown.

Starch agar: Growth excellent. Aerial mycelium white to gray. Hydrolysis. Soluble pigment faint brown.

Gelatin: Grayish-white ring and submerged pellicle. Pigmented layer dark brown; becomes greenish-brown when shaken. Medium liquefaction.

Milk: Faint grayish-white tinge. Slow peptonization turning dark purple at first and later brownish-purple. Reaction acid, pH 6.4.

Nitrate reduction: Strong.

Potato: Growth heavy, wrinkled. Aerial mycelium grayish-black. Potato darkened.

Cellulose: No decomposition.

Antagonistic properties: Produces an antibiotic, D-4-amino-3-isoxazolidone (novobiocin).

Habitat: Soil.

99. *Streptomyces gedanensis* (Löhlein, 1909) Müller, 1950 (Löhlein, M. Z. Hyg. Infektionskrankh. **63:** 1–16, 1909; Müller, R. Medizinsche Mikrobiologie, 4th ed. 1950, Urban & Schwarzenberg, Münich, p. 294).

Morphology: Aerial mycelium consists of short, gnarled hyphae. Spores short, oval to spherical.

Synthetic agar: Growth dark to almost black, with dark reverse. Aerial mycelium abundant, mouse-gray. No soluble pigment.

Nutrient agar: Growth thin, colorless. No aerial mycelium. No soluble pigment.

Glucose agar: Growth cream-colored, becoming black with light margin. Aerial mycelium abundant, mouse-gray.

Potato: Growth lichenoid, cream-colored to brownish. No aerial mycelium. No soluble pigment.

Gelatin: Growth thin, flaky. No soluble pigment. Rapid liquefaction.

Milk: Surface ring cream-colored. No peptonization.

Starch media: Growth yellowish to cream-colored. Aerial mycelium light gray. Hydrolysis strong.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Source: Sputum of patient with chronic lung disease.

Type culture: IMRU 3417.

100. *Streptomyces gelaticus* (Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. Soil Sci. **8:** 165, 1919).

Synonym: *Streptomyces hepaticus.*

Morphology: Sporophores produce open spirals. According to Anderson *et al.* (1956), the organism does not produce spirals.

Sucrose nitrate agar: Growth colorless, spreading, chiefly deep into the medium. Aerial mycelium thin, white, turning grayish.

Nutrient agar: Growth only on surface, wrinkled, cream-colored.

Glucose agar: Growth abundant, spreading, white.

Potato: Growth abundant, much wrinkled, greenish, becoming black with yellowish margin.

Gelatin: Produces flaky, cream-colored sediment. Good liquefaction.

Milk: Pinkish ring. Coagulation and peptonization.

Starch: Growth thin, spreading, cream-colored. Hydrolysis.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 25°C.

Antagonistic properties: Produces elaiomycin.

Habitat: Soil.

Remarks: Various related forms have been described by Gause *et al.* (1957); these include *A. griseorubens*, *A. rubiginosus*, and *A. atroolivaceus.* Krassilnikov (1959) considers this organism as belonging to the *S. albus* group.

Type culture: IMRU 3323.

101. *Streptomyces glaucus* (Lehmann and Schütze *emend.* Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 46, 1941).

Morphology: Sporophores form compact spirals with 3 to 5 turns. Spores oval to spherical, 1.0 by 0.8 μ.

Sucrose nitrate agar: Growth colorless; soluble pigment brown. Aerial mycelium at first white, then becoming bright green.

Nutrient agar: Growth heavy. Aerial mycelium green.

Potato: Growth heavy. Aerial mycelium velvety, green.

Gelatin: Liquefaction slow. Melanin-negative.

Milk: Peptonization slow, with prior coagulation by some strains.

Starch: Hydrolysis rapid.

Cellulose: Growth good.

Nitrate reduction: Positive.

Sucrose: Poor inversion.

Paraffin: Growth good.

Antagonistic properties: All strains strongly antagonistic.

Habitat: Soil.

Remarks: Numerous cultures belonging to this organism or closely related to it have

been described under a variety of different names. It is sufficient to mention *S. caelestis*, which produces an antibiotic, celesticetin, described by DeBoer *et al.* (1954) and *A. glaucescens*, together with a variety *badius*, described by Gause *et al.* (1957).

102. *Streptomyces globisporus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 48, 1941).

Morphology: Sporophores straight or wavy, often gathered in clusters or tufts; no spirals. Spores oval (1.2 to 1.4 by 1.8 to 2.0 μ) or spherical (0.9 to 1.4 μ).

Starch-KNO_3 agar: Growth abundant, colorless. Aerial mycelium light yellow to greenish-yellow with pinkish tinge. No soluble pigment.

Glucose-peptone agar: Growth colorless or greenish. Aerial mycelium creamy, seldom greenish-yellow. Soluble pigment absent or faint yellowish.

Gelatin: Rapid liquefaction. Soluble pigment absent or light brownish.

Potato: Growth colorless or brownish. Aerial mycelium greenish-yellow. Plug brownish or colorless.

Milk: No coagulation; rapid peptonization.

Starch: Weak hydrolysis.

Invertase: None.

Nitrate: Reduced to nitrite.

Cellulose: No or poor growth.

Antagonistic properties: Some strains suppress gram-positive bacteria.

Habitat: Soil.

Remarks: Krassilnikov recognized several substrains of the species on the basis of milk coagulation, proteolysis, and pigmentation of aerial mycelium. It is sufficient to mention *A. globisporus vulgaris*, *A. globisporus griseus*, *A. globisporus lactis*, *A. globisporus diastaticus*, *A. globisporus flavcolus*, *A. globisporus circulatus*, *A. globisporus scabies*, and *A. globisporus albus*. This heterogeneous collection is most unfortunate, since these "species" show differences in color of aerial mycelium, in formation of soluble pigments, etc.

Krassilnikov (1949) considered the streptomycin-producing organism as a variety of this species, designating it at first as *A. globisporus streptomycini*, and later as *A. streptomycini; A. griseus* Krainsky was distinguished from this species on the basis of the fact that the sporophores of the latter exhibited spiral formation. This again was the cause of much confusion in nomenclature of the streptomycin-producing organism in the literature of the Soviet Union.

Later, Krassilnikov (1958) divided the *A. globisporus* group, on the basis of antagonistic effects, into a number of subgroups, including *A. vulgaris*, *A. toxicus*, *A. levoris*, *A. bacillaris*, *A. fluorescens*, *A. raffinosus*, *A. longisporus*, and *A. grisinus*.

Gause *et al.* (1957) described *A. globisporus* in the series "*helvolus*," comprising the *S. griseus* group; they also listed several additional forms belonging to *S. globisporus* under the names *A. caucasicus* and *A. cyanofuscatus*. The above description is based upon the comparison made by Gause *et al.* of six cultures and Krassilnikov's authentic strain.

103. *Streptomyces globosus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 58, 1941).

Morphology: Sporophores straight, short, slightly branched, wavy. Spores spherical to oval.

Agar media: Substrate growth brown to dark brown. Aerial mycelium dark gray, velvety. Soluble pigment dark brown.

Gelatin: Weak liquefaction. No soluble pigment. Melanin formation questioned by Hoffmann (1958).

Potato: Soluble pigment red-brown (Hoffmann, 1958).

Milk: Questionable coagulation; good peptonization.

Starch: Hydrolysis.

Cellulose: Good growth.

Sucrose: No inversion.

Production of H_2S: Positive.

Antagonistic properties: No activity.

Habitat: Soil, food products, potatoes.

Type culture: IMRU 3736.

104. *Streptomyces gougeroti* (Duché, 1934) Waksman and Henrici, 1948 (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Morphology: Aerial hyphae short, gnarled. Spores oval.

Glucose nitrate agar: Growth slow as punctiform colonies; cream-colored with smooth edge. No aerial mycelium. No soluble pigment.

Glucose-asparagine agar: Growth colorless to yellowish. Aerial mycelium thin, white. No soluble pigment.

Nutrient agar: Growth cream-colored with brownish reverse. Aerial mycelium thin, white. Soluble pigment faint yellowish.

Potato: Growth slow, greenish tinged. Aerial mycelium thin, white. No soluble pigment.

Gelatin: Surface growth heavy, cream-colored. Aerial mycelium thin, white. Liquefaction strong. Melanin-negative.

Milk: Growth cream-colored. Aerial mycelium thin, white. Peptonization rapid.

Coagulated serum: Growth cream-colored. Aerial mycelium white. Liquefaction rapid.

Starch: Hydrolysis rapid.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Antagonistic properties: Active against fungi.

Remarks: This culture is believed to be intermediate between *S. albus*, with its abundant aerial mycelium, and *S. almquisti*, with its very scant aerial mycelium.

Type culture: IMRU 3590.

105. *Streptomyces gracilis* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl Biol. **13:** 580, 1926).

Morphology: Sporophores form spirals. Spores oval or spherical, 0.8 to 0.9 by 0.8 μ.

Sucrose nitrate agar: Growth fern-like, pale gray. Aerial mycelium scant, gray to buff. Soluble pigment cream-colored.

Nutrient potato agar: Growth vinaceous-buff to dark brown or almost black. Aerial mycelium gray. Soluble pigment light golden brown.

Potato: Aerial mycelium abundant, olive-gray to buff. Plug pigmented light brown.

Gelatin: Growth gray. Aerial mycelium white. Liquefaction rapid. Soluble pigment pink to dark golden brown.

Milk: Surface growth good. Aerial mycelium white in the form of a ring and specks on surface. Coagulation slow, followed by rapid peptonization.

Starch: Positive hydrolysis.

Nitrate reduction: Positive.

Tyrosinase reaction: Negative.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab.

106. *Streptomyces griseinus* Waksman (Reynolds, D. M. and Waksman, S. A. J. Bacteriol., **55:** 739–751, 1948; Okami, Y. J. Antibiotics (Japan) **3:** 95–97, 1950).

Morphology: Straight sporophores produced in clusters or tufts, without spirals. Spores rod-shaped, 1.0 to 1.8 by 0.8 to 1.0 μ.

Sucrose nitrate agar: Substrate growth wrinkled, reverse cream-colored to brownish. Aerial mycelium white to cream-colored with light greenish tinge (lesser tendency to grass-green coloration, more of a cream-color). No soluble pigment.

Starch agar: Colorless to cream-colored growth. Aerial mycelium grayish-olive. Hydrolysis rapid.

Potato: Growth wrinkled, yellowish-white. Aerial mycelium grayish-white with olive tinge.

Gelatin: Growth cream-colored with brownish tinge. Aerial mycelium absent, or scant, white. Liquefaction rapid.

Milk: Growth cream-colored. Coagulation and peptonization.

Tyrosine agar: No pigment produced.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Carbon utilization: Okami (1950) reported that the grisein-producing organism (*S. griseinus*) grows more readily in synthetic media containing glucose, glycerol, and sucrose than the streptomycin-producing *S. griseus*. According to Benedict *et al.* (1955), the former utilizes xylose, L-arabinose, and rhamnose, but *S. griseus* utilizes only xylose.

Phage sensitivity: Not sensitive to phages effective against *S. griseus* strains.

Pigments: No soluble pigments on calcium malate or succinate media, whereas *S. griseus* forms green and yellow pigments on these media, according to Benedict and Lindenfelser (1951).

Antagonistic properties: Produces the antibiotic grisein. Albomycin, produced by *A. subtropicus* (Gause, 1955), is an identical or closely related compound (Waksman, 1957; Thrum, 1957).

Remarks: *S. griseus* and *S. griseinus* show other striking differences. There are some close resemblances between these and the viomycin-producing cultures. Thus, on tyrosine-starch agar, certain *S. griseus* strains form a dark pigment in the agar, whereas *S. griseinus* strains resemble the viomycin-producing cultures by not forming this pigment. *S. griseinus* and the viomycin group grow well on $NaNO_3$, but *S. griseus* utilizes this compound poorly. *A. subtropicus*, described by Kudrina and Kochetkova (1958), is closely related to, if not identical with *S. griseinus*.

Type culture: IMRU 3478.

107. *Streptomyces griseobrunneus* Waksman, 1919 (Waksman, S. A. Soil Sci. **8:** 125–127, 1919).

Morphology: Sporophores usually straight on most media; often a few short, open spirals are formed; tufts are produced on certain media. Spores oval-shaped.

Sucrose nitrate agar: Growth cream-colored to yellowish-brown. Aerial mycelium appears early; powdery, olive-buff to water-green. No soluble pigment.

Glycerol-calcium malate agar: Growth cream-colored. Aerial mycelium water-green in color.

Glucose-asparagine agar: Growth yellowish-brown. Aerial mycelium pale olive-buff. No soluble pigment.

Nutrient agar: Growth cream-colored, becoming brown. Aerial mycelium abundant, white. Soluble pigment brown.

Starch agar: Growth cream-colored to yellowish. Aerial mycelium white. Good hydrolysis.

Egg media: Growth cream-colored with brownish tinge. Aerial mycelium olive-buff. Soluble pigment purple.

Potato: Growth brownish. Aerial mycelium white, turning olive-buff. Soluble pigment faintly brown.

Gelatin: Growth cream-colored, turning brown. Aerial mycelium white. Soluble pigment deep brown. Medium liquefaction.

Milk: No coagulation; rapid peptonization.

Nitrate reduction: Positive.

Sucrose: No inversion.

Cellulose: Good growth.

Habitat: Sewage.

Remarks: This organism had been described by Waksman (1919) as *Actinomyces* 218, but never named before. It was said to be closely related to *S. griseus*, differing from it by lesser proteolysis and production of a brown pigment on protein media.

Type culture: IMRU 3068.

108. *Streptomyces griseocarneus* Benedict *et al.*, 1951 (Benedict, R. G., Lindenfelser, L. A., Stodola, F. H., and Traufler, D. H. J. Bacteriol. **62:** 487–497, 1951; see also

Grundy, W. E. Antibiotics & Chemotherapy **1:** 309–317, 1951).

Morphology: Sporulation occurs best on a carbon-free salt agar, to which 0.5 per cent soluble starch has been added. Sporophores straight, forming no spirals. Spores coccoid to oval, 1.1 to 1.6 by 0.7 to 1.1 μ.

Agar media: Aerial mycelium on some media powdery, becoming gray, but no sporulation. When sporulation occurs the mycelium becomes light pink.

Sucrose nitrate agar: Growth limited, white. Aerial mycelium white, no sporulation.

Glucose-asparagine agar: Growth moderate. Aerial mycelium powdery, white, no sporulation.

Calcium malate agar: Growth moderate, white. Aerial mycelium white, no sporulation.

Nutrient agar: Growth moderate, cream-colored. No aerial mycelium. Soluble pigment light yellow-brown.

Oatmeal agar: Growth luxuriant, brown. Aerial mycelium abundant, fluffy, white; no sporulation. No soluble pigment.

Potato: Growth cream-colored. Aerial mycelium gray. Soluble pigment light brown, turning dark brown.

Gelatin: Growth cream-colored to brown. Rapid liquefaction. Soluble pigment dark brown.

Milk: Growth dark brown to black. No coagulation; rapid peptonization. Soluble pigment brown.

Starch: Hydrolysis.

Carbon utilization: Glucose, dextrin, starch, glycerol, calcium malate, and sodium succinate rapidly utilized. Mannose, maltose, inositol, and sodium acetate utilized slowly. Xylose, galactose, sorbose, sucrose, cellobiose, melibiose, lactose, mannitol, sorbitol, sodium citrate, and potassium sodium tartrate not utilized.

Nitrate reduction: Negative.

Production of H_2S: Positive.

Antagonistic properties: Produces hydroxystreptomycin.

Type culture: IMRU 3557; ATCC 12,628.

109. *Streptomyces griseochromogenes* Fukunaga *et al.*, 1955 (Fukunaga, K., Misato, T. Ishii, I., and Asakawa, M. Bull. Agr. Chem Soc. Japan **19:** 181–188, 1955).

Morphology: Sporophores form closed spirals on starch agar; there are no spirals, or only curling tips, formed on sucrose nitrate and glucose-asparagine agars. Spores spherical or oval, about 1.0 to 1.5 μ.

Sucrose nitrate agar: Growth spreading, orange-cinnamon. Aerial mycelium white or light neutral gray. No soluble pigment.

Glucose-asparagine agar: Growth restricted, ivory-yellow, penetrating into the medium. No aerial mycelium, later white. No soluble pigment.

Nutrient agar: Growth restricted, opalescent. No aerial mycelium. Soluble pigment brown.

Potato: Growth abundant, wrinkled, snuff-brown. Aerial mycelium white to mouse-gray. Color of plug dark brown to black around growth.

Gelatin: Growth wrinkled, yellowish in liquefied portion. Aerial mycelium white, scant. Liquefaction medium. Soluble pigment dark brown to black.

Milk: Growth as surface ring, brown. No coagulation; peptonization begins in 8 days at 37°C; not completed in 21 days.

Nitrate reduction: Positive.

Cellulose: No growth.

Tyrosine agar: Growth orange-colored. No soluble pigment.

Invertase: Positive.

Carbon utilization: Glucose, D-fructose, D-galactose, maltose, lactose, raffinose, D-mannitol, DL-inositol utilized. Rhamnose, inulin, D-sorbitol, dulcitol, salicin, sodium acetate, sodium citrate, sodium succinate not utilized.

Antagonistic properties: Produces blasticidins A, B, and C, active against fungi.

Remarks: *S. griseochromogenes* belongs to the group of chromogenic actinomycetes. *S. resistomycificus* differs from *S. griseochromogenes* in the color of its aerial mycelium observed on various media, and also in that it produces an aerial mycelium on nutrient agar and a soluble pigment in glucose-asparagine agar. *S. mirabilis* has a different form of aerial mycelium and a different optimum temperature. *S. flavochromogenes* produces an aerial mycelium on nutrient agar and a grayish soluble pigment. *S. olivochromogenes* assumes a dark brown or black color of growth and shows an alkaline reaction in milk medium. *S. diastatochromogenes* is quite similar to *S. griseochromogenes* in the appearance of its growth on several media, but differs from it by producing a white or gray aerial mycelium on nutrient agar, and also by producing tyrosinase.

110. *Streptomyces griseoflavus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41:** 684, 1914).

Morphology: Sporophores straight, monopodially branched; no curvatures and no spirals produced. Spores oblong, 1.0 to 1.2 μ, covered with short spines. (According to Ettlinger *et al.* (1958), open, regular spirals are formed.)

Sucrose nitrate agar: Growth reddish-brown to orange. Aerial mycelium gray to yellowish-gray. Faint greenish-yellow soluble pigment.

Glucose-asparagine agar: Growth citron-yellow. Aerial mycelium powdery, greenish-yellow changing to gray (Hoffmann, 1958).

Calcium malate agar: Growth yellowish-green-gray.

Nutrient agar: Growth cream-colored, covered with white to gray aerial mycelium. Soluble pigment absent or, according to Hoffmann (1958), greenish-gray.

Starch agar: Growth cream-colored with brownish center. Aerial mycelium absent or powdery, gray. Hydrolysis limited.

Potato: Growth lichenoid, yellowish. Aerial mycelium powdery, white to gray. Melanin-negative.

Gelatin: Growth cream-colored to brownish, covered with white to yellowish-gray aerial mycelium. Positive liquefaction. Melanin-negative.

Milk: Growth cream-colored to yellowish; aerial mycelium thin, white. No coagulation; rapid peptonization.

Cellulose: Growth good. Greenish-yellow soluble pigment, according to Hoffmann (1958).

Nitrate: Strong reduction to nitrite.

Production of H_2S: Negative.

Invertase: Negative.

Antagonistic properties: According to Waga (1953), a member of this group produced an antibiotic, griseoflavin; this antibiotic was later (Kuroya *et al.*, 1958) found to be identical with novobiocin. Another antibiotic, grisamine, has also been reported.

Remarks: According to Jensen (1930), the species is characterized by the grayish-yellow color of its aerial mycelium, which never assumes the distinct green shade of *S. griseus*. A detailed study of the life cycle of this organism has been made by Saito and Ikeda (1958). They found that between the primary (vegetative phase) and the secondary (sporulation phase) mycelium, there may be a transitional stage which comprises "nests," "swollen bodies," and "clubs," corresponding to the "initial cells" reported in the literature.

111. *Streptomyces griscolus* (Waksman, 1923) Waksman and Henrici, 1948 (Waksman, S. A. *Actinomyces* 96. Soil Sci. **8:** 121, 1919).

Morphology: Sporophores short, straight, without spirals, some curling found on side branches. Spores spherical to oval-shaped to cylindrical.

Sucrose nitrate agar: Growth colorless, thin, spreading, chiefly in the medium. Aerial mycelium at first gray, later becoming

pallid neutral gray, with yellowish tone. Faint brownish soluble pigment.

Malate-glycerol agar: Growth brownish. Aerial mycelium light mouse-gray. Soluble pigment faint brownish.

Glucose-asparagine agar: Growth cream-colored, turning dark. Aerial mycelium deep dull gray. No soluble pigment.

Nutrient agar: Brownish growth, with smooth surface. Aerial mycelium white with gray tinge. Soluble pigment brown. Melanin-negative.

Potato: Growth cream-colored, becoming black. Aerial mycelium white with greenish tinge. Soluble pigment brown to black.

Gelatin: Yellowish flaky pellicle and sediment. Aerial mycelium white. Gradual liquefaction. Faint browning of medium.

Milk: Growth abundant, pink pellicle. Slow coagulation and good peptonization.

Starch media: Growth grayish-brown with dark ring. Aerial mycelium gray. Slight hydrolysis of starch.

Cellulose: Scant growth.

Invertase: Negative.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 25°C.

Antagonistic properties: Some strains show considerable activity against various bacteria. Several antibiotics (phagomycin, fermicidin, anisomycin, oxytetracycline, griscomycin) were isolated from cultures described as strains of *S. griseolus*.

Habitat: Soil.

Remarks: Ettlinger *et al.* (1958) considered this organism as related to *S. olivaceus*. Krassilnikov (1949) considered it as a strain of *A. candidus*. Hoffmann (1958) isolated a strain of this species from potato scab. Although this organism is usually described as melanin-negative, Krassilnikov (1941) and Hoffmann consider it as melanin-positive.

Type culture: IMRU 3325.

112. *Streptomyces griseoluteus* Umezawa *et al.*, 1951 (Umezawa, H., Hayano, S., Maeda, K., Ogata, Y., and Okami, Y. J. Antibiotics (Japan) **4:** 34–40, 1951; Okami, Y. *ibid.* **5:** 477–480, 1952).

Morphology: Sporophores with monopodial and irregular branching, flexible and hooked. Spores oval to cylindrical, 1.0 to 1.2 by 1.8 to 2.2 μ.

Sucrose nitrate agar: Growth thin, colorless to cream-colored. Margin plumose, penetrating into medium. Aerial mycelium powdery, grayish-white to light drab. Soluble pigment absent or yellowish-brown.

Glucose-asparagine agar: Growth wrinkled, cream-colored. Aerial mycelium thin, white. Pigment reddish-brown.

Nutrient agar: Growth wrinkled, transparent. Aerial mycelium thin, white, powdery. Soluble pigment absent or yellowish-brown.

Potato: Growth abundant, wrinkled, cream-colored. Aerial mycelium dusty white, thin. Plug becoming slightly brownish.

Gelatin: No growth.

Milk: Surface ring cream-colored. Aerial mycelium in form of white patches.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Antagonistic properties: Produces griseolutein.

Type culture: IMRU 3674; 3729.

113. *Streptomyces griseoplanus* Backus *et al.*, 1957 (Backus, E. J., Tresner, H. D., and Campbell, T. H. Antibiotics & Chemotherapy **7:** 532–541, 1957).

Morphology: Sporophores arise as tangled and curved and often loosely spiraled chains of spores. Spores globose to elliptical, 0.6 to 1.2 by 1.2 to 1.5 μ (Fig. 40).

Sucrose nitrate agar: Growth colorless. Aerial mycelium scant, white to gray.

Glucose-asparagine agar: Growth gray to light pinkish. No aerial mycelium.

Nutrient agar: Growth ivory-yellow. No aerial mycelium. Melanin-negative.

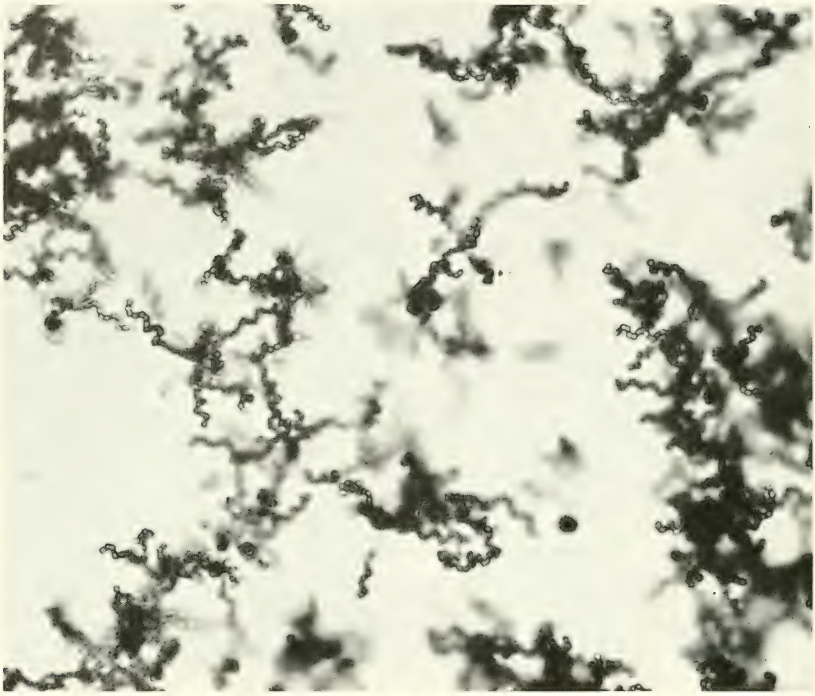

FIGURE 40. Sporophores of *S. griseoplanus* (Reproduced from: Backus, E. J. *et al.* Antibiotics & Chemotherapy **7**: 537, 1957).

Starch agar: Growth colorless to yellowish. Aerial mycelium scant, white.

Potato plug: Growth light brownish. Aerial mycelium scant, white to gray. Apex of plug browned.

Milk: No growth.

Cellulose: No growth.

Production of H_2S: Negative.

Carbon utilization: Extremely limited. with ammonium sulfate as source of nitrogen. With aspartic acid, organism utilizes L-arabinose, D-xylose, and glucose. Fair to moderate growth on lactose, D-raffinose, D-trehalose, and salicin. Sucrose, D-fructose, D-mannitol, *i*-inositol, adonitol, D-melezitose, L-rhamnose, esculin,D-melibiose, and dextrin utilized poorly or not at all.

Antagonistic properties: Produces antibiotic alazopeptin.

Habitat: Grassland soil.

Remarks: This organism is closely related to *S. flavogriseus*.

114. *Streptomyces griseoruber* Yamaguchi and Saburi, 1955 (Yamaguchi, T. and Saburi, Y. J. Gen. Appl. Microbiol. **1**: 201–235, 1955).

Morphology: Aerial hyphae produce open and closed spirals on sucrose nitrate and on starch agars. Spores short, cylindrical, 0.5 to 0.9 by 0.9 to 1.2 μ.

Sucrose nitrate agar: Growth reddish-orange when freshly isolated, but changes to colorless or whitish on repeated transfer. Aerial mycelium powdery, drab-gray. No soluble pigment.

Calcium malate agar: Growth at first glossy, pinkish-gray, later becoming dull purplish. Aerial mycelium powdery, olive-gray. Soluble pigment absent or faint yellowish-brown.

Nutrient agar: Growth at first light olive-gray, later becoming brown. Aerial mycelium absent or scanty, white. Soluble pigment brown.

Starch agar: Growth light reddish-orange to reddish-purple. Aerial mycelium powdery, olive-gray. Soluble pigment faint yellow to faint yellowish-pink. Strong hydrolysis.

Potato: Growth wrinkled, at first olive-gray to dark yellowish-brown, later becoming dark reddish or black. Aerial mycelium absent or scanty, white. Soluble pigment deep purple to black.

Gelatin: Growth light yellowish-brown on surface. No aerial mycelium. Soluble pigment brown to light yellowish-green. Liquefaction weak to medium.

Milk: Growth deep brown at 37°C, but cream-colored to light yellowish-brown, and occasionally with pinkish tone at 25°C. Soluble pigment grayish-yellow-brown at 37°C, but sometimes faint yellowish-brown with pinkish shade at 25°C. Coagulation begins in 3 days, followed by peptonization.

Cellulose: No growth.

Carbon utilization: Utilizes D-xylose, L-arabinose, L-rhamnose, lactose, inositol, salicin, sodium acetate; does not utilize sucrose, raffinose, inulin, mannitol, sorbitol, citrate, and succinate.

Antagonistic properties: Active against gram-positive and acid-fast bacteria; possesses antitrichomonal activity.

Remarks: Related to the *S. ruber* group and to *S. erythrochromogenes.*

115. *Streptomyces griseoviridis* Anderson *et al.*, 1956 (Anderson, L. E., Ehrlich, J., Sun, S. H., and Burkholder, P. R. Antibiotics & Chemotherapy **6:** 100–115, 1956).

Not *S. griseoviridus.*

Morphology: Sporophores straight or curved, with open and closed spirals on lateral branches. Spores spherical to ovoid, 0.6 to 1.5 by 0.8 to 2.1 μ.

Starch-ammonium sulfate agar: Growth tan-gray to black. Aerial mycelium tan to light brown.

Glycerol-asparagine agar: Growth light yellow to gray. Aerial mycelium pink-tan to gray-green.

Calcium malate agar: Growth yellow-tan to gray. Aerial mycelium light brown. Slight hydrolysis of starch.

Nutrient agar: Growth yellow-tan to green-gray to brown. Aerial mycelium light gray-pink to light gray-green. Soluble pigment light brown.

Glucose-tryptone agar: Growth light brown to red-brown to dark brown or black. Aerial mycelium pink-gray to light brown to green-brown. Soluble pigment brown; red-brown near growth.

Gelatin: Fairly rapid liquefaction. Soluble pigment light brown to dark brown.

Milk: Peptonization.

Starch: Hydrolysis.

Carbon utilization: Utilizes arabinose, cellobiose, dextrin, galactose, glucose, glycerol, lactose, levulose, maltose, mannitol, mannose, rhamnose, starch, trehalose, and xylose. Does not utilize esculin, adonitol, dulcitol, *i*-inositol, inulin, melezitose, melibiose, raffinose, salicin, sorbitol, and sucrose.

Antagonistic properties: A source of griseoviridin and viridogrisein (etamycin).

Habitat: Texas soil.

Type culture: IMRU 3735.

116. *Streptomyces griseus* Waksman and Henrici, 1948 (Waksman and Henrici, Bergey's Manual, 6th ed. 1948, p. 948; Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 119–120, 1916; Waksman, S. A., Reilly,

H. C., and Harris, D. A. J. Bacteriol. **56:** 259, 1948; Waksman, S. A. Proc. Natl. Acad. Sci. U. S. **45:** 1043–1047, 1959).

Synonyms: *Actinomyces globisporus* Krassilnikov, 1941. *Actinomyces globisporus* subsp. *streptomycini* (Waksman) Krassilnikov, 1949. *Actinomyces streptomycini* Krassilnikov, 1957.

Morphology: Sporophores straight, produced in tufts (Fig. 41). Spores spherical to oval, 0.8 by 0.8 to 1.7 μ; surface smooth (Pl. II i).

Sucrose nitrate agar: Growth thin, spreading, colorless, becoming olive-buff. Aerial mycelium thick, powdery, water-green. Pigment insoluble.

Nutrient agar: Growth abundant, almost transparent, cream-colored. Aerial mycelium powdery, white to light gray. No soluble pigment.

Glucose agar: Growth elevated in center, radiate, cream-colored to orange, erose margin.

FIGURE 41. Substrate and aerial mycelium of *S. griseus*.

Starch media: Growth thin, spreading, transparent. Hydrolysis strong.

Tyrosine agar: Dark pigment often produced.

Potato: Growth wrinkled, yellowish to brownish, covered with white, powdery aerial mycelium.

Gelatin: Greenish-yellow or cream-colored surface growth with brownish tinge. Rapid liquefaction.

Milk: Cream-colored ring; coagulation with rapid peptonization, becoming alkaline.

Cellulose: Scant to fair growth.

Nitrate reduction: Positive.

Pigments: Produces green or yellow soluble pigment on calcium malate and succinate media.

Production of H_2S: Negative.

Carbon sources: See *S. griseinus*.

Antagonistic properties: Strongly antagonistic. Produces antibiotic streptomycin, active against a large number of bacteria and actinomycetes, but not against most fungi or viruses; also produces cycloheximide, active upon fungi. Resistant to streptomycin-producing organisms and to streptomycin.

Remarks: An extensive literature has accumulated on the nature of this organism (Koreniako and Nikitina, 1959), on its phage sensitivity (Koerber *et al.*, 1950), antibiotic production (Waksman, 1949), etc.

Habitat: Soils, river muds, throat of chicken.

Type culture: IMRU 3463.

117. *Streptomyces hachijoensis* Yamaguchi, 1954 (Yamaguchi, T. J. Antibiotics (Japan) **7A:** 10–14, 1954).

Morphology: Aerial hyphae short, straight, 0.6 to 1.2 μ. Secondary verticils produced. Spores cylindrical, 0.8 to 1.0 by 1.5 to 1.8 μ.

Sucrose nitrate agar: Growth restricted, colorless; reverse yellowish. Aerial mycelium white, changing to pinkish-buff. No soluble pigment.

Calcium malate agar: Growth colorless to

yellow. Aerial mycelium white to pale pinkish-buff. Soluble pigment absent.

Nutrient agar: Growth cream-colored, wrinkled. Aerial mycelium powdery, shade of pale ochraceous-buff. No soluble pigment.

Potato: Growth cream-colored to yellowish, wrinkled, raised. Aerial mycelium white. Soluble pigment around growth faint purplish.

Blood agar: Growth yellow to brownish-yellow. Aerial mycelium flocculent, white. Soluble pigment dark. Positive hemolysis.

Gelatin: Growth yellow to brown. No aerial mycelium. Rapid liquefaction.

Milk: Surface ring yellow to brown. Aerial mycelium in form of white patches. Soluble pigment pinkish to orange. Coagulation followed by peptonization.

Nitrate reduction: Negative.

Cellulose: No growth.

Antagonistic properties: Produces an antifungal agent, trichomycin, a member of the candicidin group of antibiotics.

Remarks: Resembles *S. rubrireticuli*. Blinov (1958) described a variety (*fuscatus*) of this species, as a producer of candicidin-type antibiotics.

118. *Streptomyces halstedii* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 124, 1916; **8:** 121, 1919).

Morphology: Sporophores form closed spirals. Spores oval or rod-shaped, 1.0 to 1.2 by 1.2 to 1.8 μ.

Sucrose nitrate agar: Substrate growth abundant, spreading, raised, at first light colored, becoming dark to almost black. Aerial mycelium white, turning dull gray. No soluble pigment.

Glycerol malate agar: Growth dark. Aerial mycelium deep mouse-gray.

Nutrient agar: Growth restricted, wrinkled, cream-colored. No aerial mycelium. Melanin-negative.

Glucose-asparagine agar: Growth wrinkled, center elevated, edge lichenoid, colorless, becoming brown. No aerial mycelium.

Potato: Growth abundant, moist, wrinkled, cream-colored with green tinge.

Gelatin: Small, cream-colored masses of growth in bottom of tube. Rapid liquefaction. No soluble pigment.

Milk: Cream-colored ring. Coagulation and slow peptonization.

Starch media: Growth abundant, glossy, brownish. No aerial mycelium. Rapid hydrolysis.

Cellulose: No growth.

Nitrate: Reduction to nitrite.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Strongly antagonistic; some strains show only antifungal activity; some strains produce carbomycin.

Habitat: Soil.

Remarks: Several closely related forms have been described. According to Ettlinger *et al.* (1958), the strains examined produce no spirals and belong to *S. olivaceus*. According to Okami and Suzuki (1958), the sporophores are wavy, seldom forming hooks or primitive spirals. Gause *et al.* (1957) described a closely related form as *A. griseoincarnatus*.

Type culture: IMRU 3328.

119. *Streptomyces hawaiiensis* Cron *et al.*, 1956 (Cron, M. J., Whitehead, D. F., Hooper, I. R., Heinemann, B., and Lein, J. Antibiotics & Chemotherapy **6:** 63–67, 1956).

Morphology: Sporophores produce spirals on some media. Spores oval, 0.6 to 0.8 by 0.7 to 1.3 μ.

Sucrose nitrate agar: Growth faint yellow. Aerial mycelium sparse, white to flesh-colored. Soluble pigment faint tan or absent.

Glucose-asparagine agar: Growth light brownish. Aerial mycelium moderate, white to gray. Soluble pigment faint tan or absent.

Nutrient agar: Growth gray with light brown reverse. Aerial mycelium limited,

wood-ash to steel-gray. Soluble pigment brown.

Potato: Growth gray. Aerial mycelium limited, gray. Soluble pigment dark brown, almost black.

Milk: No coagulation or peptonization. Slight acid reaction. Soluble pigment greenish-brown.

Gelatin: Slight liquefaction at 26°C in 19 days. Soluble pigment brown.

Starch: Slight hydrolysis in 7 days at 28–30°C.

Blood agar: No hemolysis. Soluble pigment black.

Production of H_2S: Positive.

Carbon utilization: Utilizes arabinose, rhamnose, glucose, galactose, fructose, sucrose, maltose, lactose, xylose, raffinose, cellobiose, dextrin, inulin, soluble starch, glycerol, inositol, mannitol, sodium acetate, sodium citrate, and calcium malate. Does not utilize sorbitol, dulcitol, sodium oxalate, sodium salicylate, sodium tartrate, and sodium succinate.

Antagonistic properties: Produces a polypeptide antibiotic, bryamycin.

Remarks: *S. hawaiiensis* is a chromogenic form which produces a soluble, dark brown pigment on protein media and a white to gray aerial mycelium. The organism is further characterized by spiral formation in the aerial mycelium and weak proteolytic activity in gelatin and milk.

Streptomyces phaeofaciens possesses cultural and morphological characteristics similar to those of *S. hawaiiensis*, but differs in its rapid peptonization of milk and production of an antifungal substance inactive on bacteria.

S. hawaiiensis resembles *S. aureus* in that spiral formation occurs with both cultures and both form soluble brown pigments in organic media. They differ in that *S. aureus* liquefies gelatin to a greater extent. *S. hawaiiensis* is also similar to *S. bikiniensis* in some of its cultural properties; both produce a white aerial mycelium which becomes gray-colored; the sporulation of *S. hawaiiensis* takes place in the form of spirals in its aerial mycelium, whereas *S. bikiniensis* is completely devoid of spirals and produces an alkaline reaction accompanied by hydrolysis in milk.

Type culture: ATCC 12,236.

120. *Streptomyces hiroshimensis* Shinobu, 1955 (Shinobu, R. Seibutsugakkaishi **6:** 43–46, 1955).

Morphology: Sporophores produce verticils of the Nitella type, both primary and secondary. No spirals. Spores elliptical to oval, 0.8 to 1.2 μ (Pl. V, Gb).

Sucrose nitrate agar: Growth poor, restricted, pink. Aerial mycelium scant, pale pink to pinkish-white.

Calcium malate agar: Growth slow. Aerial mycelium pale cinnamon-pink. Soluble pigment brownish.

Glucose-asparagine agar: Growth good, reddish-pink. Aerial mycelium pink to purplish-pink. Soluble pigment usually absent, sometimes pale brown.

Nutrient agar: Growth reddish-brown. Aerial mycelium absent, or scant, pale pink to pinkish-white. Soluble pigment brownish-orange.

Starch agar: Growth red to purplish-red. Aerial mycelium pink to pale pink.

Potato plug: Growth deep pinkish-red to brownish-black. Aerial mycelium scant, pinkish-white. Soluble pigment brownish-black.

Gelatin: Growth pale reddish-brown. Aerial mycelium absent, or scant, pinkish-white. Soluble pigment pale reddish-brown. Rapid liquefaction.

Milk: Growth deep pinkish-red. Aerial mycelium pinkish-white to pink. Soluble pigment brown with reddish tinge. No coagulation; rapid peptonization.

Nitrate reduction: Strong.

Starch: Rapid hydrolysis.

Tyrosinase reaction: Positive.

Cellulose: Not attacked.

Carbon utilization: Fructose and inositol well utilized; xylose, rhamnose, sucrose, lactose, raffinose, and mannitol not utilized; galactose and trehalose slightly utilized.

Antagonistic properties: Inhibits growth of gram-positive bacteria and fungi.

Source: Isolated from soil in Hiroshima, Japan.

Remarks: Resembles *S. rubrireticuli*.

121. *Streptomyces hirsutus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 344, 1958).

Morphology: Sporophores long, monopodially branched; short open spirals with about three coils are produced. Spores covered with narrow, long spines (Pl. II, k).

Glycerol nitrate agar: Growth colorless. Aerial mycelium at first milky white, later leek-green.

Glucose-asparagine agar: Growth colorless. No aerial mycelium.

Glycerol malate agar: Growth at first milky white; later covered with aerial mycelium gradually colored leek-green.

Starch-KNO_3 agar: Growth milky white. Aerial mycelium leek-green. Starch hydrolyzed.

Gelatin: Growth whitish-yellow, covered with light green aerial mycelium. Slow liquefaction. No soluble pigment. Melanin-negative.

Potato: Growth colorless. Aerial mycelium at first white, later leek-green.

Milk: Pellicle heavy, light yellow. Aerial mycelium white-gray. Rapid coagulation; no peptonization.

Antagonistic properties: None.

Habitat: Soil in Switzerland.

Remarks: Some of the cultures described by Gause *et al.* (1957), such as *A. acrimycini* and *A. acrimycini* var. *globosus*, are closely related to this organism.

122. *Streptomyces hominis* (Bostroem 1890; *emend.* Waksman, 1919) Waksman and Henrici, 1948. (Bostroem, E. Beitr. pathol. Anat. allgem. Pathol. **9:** 1–240, 1890; Waksman, S. A. Soil Sci. **8:** 129–130, 1919.

Synonyms: *Streptothrix hominis* Foulerton, 1899. *Oospora hominis* Ridet, 1911.

Morphology: Sporophores straight. A few dextrorse spirals on glycerol synthetic media.

Sucrose nitrate agar: Growth white with shade of yellow, turning brownish with age. Aerial mycelium white with olive tinge. No soluble pigment.

Glycerol malate agar: Growth yellowish. Aerial mycelium with olive-green tinge.

Nutrient agar: Growth yellowish. Aerial mycelium white. No soluble pigment.

Starch: Hydrolysis good.

Potato: Growth yellowish to orange, becoming brown. Aerial mycelium white. Color of plug unchanged, later becoming brown.

Gelatin: Growth cream-colored. No aerial mycelium. No soluble pigment.

Milk: Rapid coagulation and peptonization.

Nitrate reduction: Positive.

Sucrose: Not inverted.

Production of H_2S: Negative.

Habitat: Supposed to have been isolated from abscess of palm of hand; probably an air contamination. Appears to be related to the *S. griseus* series.

123. *Streptomyces humidus* Nakazawa and Shibata, 1956 (Belgian Patent 533,386. Takeda Pharmaceutical Industries Ltd., Japan, March 24, 1956; Proc. Japan Acad. **32:** 648–653, 1956).

Morphology: Sporophores form spirals. Spores oval, 1 to 1.5 by 1.2 to 2 μ.

Sucrose nitrate agar: Growth colorless. Aerial mycelium white. No soluble pigment.

Nutrient agar: Growth colorless. No aerial mycelium. No soluble pigment.

Glucose-asparagine agar: Growth colorless. Aerial mycelium white to smoke-gray or vinaceous-chamois. No soluble pigment.

Calcium malate agar: Growth colorless, becoming yellowish. Aerial mycelium white. No soluble pigment.

Starch agar: Growth colorless to cream-colored. Aerial mycelium white to pale smoke-gray. No soluble pigment.

Potato: Growth colorless. Aerial mycelium white to smoke-gray; black, moist speckles. No soluble pigment.

Gelatin: Growth colorless. No aerial mycelium. No soluble pigment. Moderate liquefaction.

Milk: Growth colorless. Aerial mycelium white. No soluble pigment. Slow peptonization.

Nitrate reduction: Positive.

Carbon utilization: D-xylose, L-arabinose, L-rhamnose, D-fructose, galactose, maltose, lactose, D-mannitol, salicin utilized. Sucrose, D-raffinose, inulin, D-sorbitol, dulcitol, *i*-inositol, sodium acetate, sodium citrate not utilized.

FIGURE 42. *S. hygroscopicus* (Reproduced from: Tresner, H. D. and Backus, E. J. Appl. Microbiol. 4: 246, 1956).

Antagonistic properties: Produces an antibiotic, acidomycin, said to be dihydrostreptomycin (see also Imamura *et al.*, 1956).

Remarks: *S. humidus* is closely related to *S. hygroscopicus*; growth of the latter on agar media and on potato is cream-colored to yellow to brown.

124. *Streptomyces hygroscopicus* (Jensen, 1931) Waksman and Henrici, 1948 (Jensen, H. L. Proc. Linnean Soc. N. S. Wales **56:** 357–358, 1931).

Morphology: Sporophores monopodially branched, with narrow compact, sinistrorse spirals, situated as dense clusters on the main stems of the sporophores (Fig. 42). Spores oval, 0.8 to 1.0 by 1.0 to 1.2 μ, smooth (Pl. II nf, Pl. IV Gb).

Sucrose nitrate agar: Growth folded, white to cream-colored, later sulfur-yellow to yellowish-gray, with golden to light orange reverse. Aerial mycelium scant, white to ash-gray. Soluble pigment golden to light orange.

Glucose-asparagine agar: Growth cream-colored to straw-yellow, later dull chrome-yellow to brownish-orange. Aerial mycelium dusty white to pale yellowish-gray; later small, moist, dark violet-gray to brownish patches produced, gradually spreading over the whole surface. Soluble pigment light yellow.

Nutrient agar: Growth wrinkled, cream-colored, later yellowish-gray with yellowish-brown reverse. Aerial mycelium scant, white.

Potato: Growth raised, wrinkled, cream-colored, later yellowish-gray to dull brownish. Aerial mycelium absent or trace of white. Melanin-negative.

Gelatin: Liquefaction slow. No soluble pigment.

Milk: No coagulation; positive peptoniza-

tion. The reaction becomes faintly acid (pH 6.0 or less).

Starch: Hydrolysis.

Cellulose: Ready decomposition by some strains.

Nitrate reduction: None with sucrose as source of energy.

Sucrose inversion: Positive.

Production of H_2S: Negative.

Antagonistic properties: Produces hygromycin, an antibiotic active against mycobacteria and roundworms (see also Pagano *et al.*, 1953).

Habitat: Soil.

Remarks: Tresner and Backus (1956) made a comprehensive study of 18 cultures representing *S. hygroscopicus* and closely related forms. They came to the conclusion that the following three properties are the fundamental characteristics of the organism: (1) sporophores terminate in tight spirals of a few to many turns, plus a clustering of such sporophores along hyphae; (2) brownish-gray (mouse-gray to benzo-brown) spore color on favorable media; (3) distinctive hygroscopic character on some agar media. The characteristic feature, not equally distinct in all strains, however, is the fact that the aerial mycelium on synthetic media becomes moistened and exhibits dark, glistening patches; when touched with a needle, these patches prove to be moist, smeary masses of spores. Shape and size of spores, color of substrate growth, formation of soluble pigments, growth on potato, growth on milk, cellulose decomposition, and carbon and nitrogen utilization were considered by Tresner and Backus as variable properties. They considered *S. platensis* and *S. endus* as closely related.

Other related forms, such as *A. kurssanovii* and *A. nigrescens*, have been described by Gause *et al.* (1957). Ettlinger *et al.* (1958) also included *S. platensis* and *S. rutgersensis* var. *castelarense* in this group. The relationship of this species to *S. violaceoniger* has been indicated by Nomi (1960b). Vavra *et al.* (1959) described a variety *decoyicus* that differed in certain minor properties. A number of other varieties of this organism have been described, such as *odoratus* (Yüntsen *et al.*, 1956) and *angustmyceticus* (Takahashi and Amano, 1954).

125. *Streptomyces intermedius* (Krüger-*emend.* Wollenweber, 1922) Waksman (Wollenweber, H. Ber. deut. botan. Ges. **39:** 26, 1922).

Morphology: Sporophores straight, wavy, frequently arranged in fascicles or clumps No spirals. Spores round to oval; 0.9 to 1.0 by 0.7 μ.

Glycerol nitrate agar: Substrate growth cream-colored to brown; sometimes dark green to greenish-brown. Aerial mycelium thin, gray to dark gray.

Glucose-asparagine agar: Growth brownish with greenish shade. Aerial mycelium dark gray. No soluble pigment.

Nutrient agar: Growth much folded, cream-colored. Aerial mycelium white. Soluble pigment faintly golden, occasionally green to olive-green; on continued cultivation, green color tends to become cream-colored to brownish.

Glucose-peptone agar: Growth good, brownish. Aerial mycelium heavy, cream-colored to dark gray. No soluble pigment.

Potato: Growth folded, brown to greenish-brown. Aerial mycelium dark gray. Soluble pigment olive-green. Melanin-negative.

Gelatin: Growth thin, colorless to faintly brown, dropping to bottom. Slow liquefaction. Greenish-brown pigment.

Milk: Surface growth heavy, cream-colored. No aerial mycelium. No coagulation, good peptonization.

Starch: Good hydrolysis.

Cellulose: Growth good, olive-green. Aerial mycelium dark gray.

Sucrose: Inversion slow.

Nitrate reduction: Limited.

Habitat: Potato scab.

Remarks: Above description was supplemented by Hoffmann (1958). Krassilnikov (1949) considers this organism as a variety of *A. cretaceus*.

Type culture: IMRU 3329.

126. *Streptomyces ipomoeae* (Person and Martin, 1940) Waksman and Henrici, 1948 (Person, L. H. and Martin, W. J. Phytopathology **30:** 913, 1940).

Morphology: Spores oval to elliptical, 0.9 to 1.3 by 1.3 to 1.8 μ.

Sucrose nitrate agar: Growth abundant, wrinkled, olive-yellow. No aerial mycelium.

Nutrient agar: Growth moderate, in form of small, shiny colonies, both on the surface and imbedded in the medium, silver-colored.

Starch agar: Growth moderate, smooth, ivory-colored. Aerial mycelium white with patches of bluish-green. No soluble pigment. Complete hydrolysis after 12 days.

Potato: Growth moderate, shiny, wrinkled, light brown. No aerial mycelium. No soluble pigment.

Gelatin: Growth scanty, after 25 days at 20°C. No aerial mycelium. No soluble pigment. Some liquefaction.

Milk: Ring on surface. No visible coagulation; positive peptonization.

Cellulose: No growth.

Nitrate reduction: Positive.

Antagonistic properties: Positive.

Habitat: Lesions caused by sweet-potato disease.

Type culture: IMRU 3476.

127. *Streptomyces kanamyceticus* Okami and Umezawa, 1957 (Umezawa, H., Ueda, M., Maeda, K., Yagishita, K., Kondo, S., Okami, Y., Utahara, R., Osato, Y., Nitta, K., and Takeuchi, T. J. Antibiotics (Japan) **10A:** 181–188, 1957).

Morphology: Sporophores flexible and hooked, no true spirals.

Glycerol nitrate agar: Growth at first colorless, later lemon-yellow. Aerial mycelium white to yellow, occasionally with a greenish or faint pinkish tinge. Soluble pigment occasionally produced, faint brown.

Glucose-asparagine agar: Growth colorless to yellow with faint pinkish-white, and yellow or hay-colored reverse. Aerial mycelium scant; develops from center of colony, white to faint pinkish-white to greenish-yellow or yellow. Soluble pigment occasionally produced, faint brown.

Calcium malate agar: Growth yellow. Aerial mycelium white-yellow.

Nutrient agar: Growth cream-colored. Aerial mycelium absent or white. No soluble pigment.

Potato: Growth wrinkled, faint yellowish-brown to yellow. Aerial mycelium scant, white. No soluble pigment. Substrate beneath growth occasionally changes to brown.

Gelatin: Liquefaction positive. No soluble pigment. Melanin-negative.

Milk: Growth colorless. Aerial mycelium absent or white. Coagulation and peptonization doubtful.

Blood agar: Growth wrinkled, grayish-reddish-brown. No aerial mycelium. No soluble pigment.

Starch: Hydrolyzed.

Carbon utilization: Utilizes arabinose, dextrin, fructose, galactose, glycerol, maltose, mannitol, mannose, raffinose, starch, sucrose, and succinate. Does not utilize inositol, inulin, lactose, rhamnose, sorbose, xylose, and acetate. Some strains grow slightly on esculin, salicin, sorbitol, and citrate.

Antagonistic properties: Produces kanamycin, an antibiotic related to the neomycin group.

Remarks: Closely related to *S. albidoflavus*, *S. lieskei*, and *S. alboflavus*. Okami *et al.* (1959a) made a detailed study of the mutants produced by this organism.

128. *Streptomyces kentuckensis* Barr and Carman, 1956 (Barr, F. S., and Carman, P. E. Antibiotics & Chemotherapy **6:** 286–289, 1956).

Morphology: Aerial mycelium thick-

walled, generally not branched; sporophores straight; do not form spirals. Spores oblong to oval, 0.5 by 0.5 to 1.5 μ. Spores produced by fragmentation of the hyphae in substrate mycelium are generally smaller than those formed from aerial hyphae.

Nutrient agar: Growth gray to yellow. No soluble pigment. Melanin-negative.

Gelatin: Slow liquefaction. No soluble pigment.

Potato: Mycelium gray. Plug darkened.

Milk: Peptonization positive.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Carbon utilization: Readily utilizes various pentoses, hexoses, disaccharides, acetate and citrate; slight utilization of rhamnose, inulin, glycerol, inositol, mannitol; does not utilize dextran or salts of oxalic, succinic, and salicylic acids.

Antagonistic properties: Effective against gram-positive and some gram-negative bacteria. Produces antibiotic raisnomycin.

Remarks: Pridham *et al.* (1958) consider this organism as a member of the *biverticillatus* group.

Type culture: ATCC 12,691.

129. *Streptomyces kimberi* (Erikson, 1935) Waksman (Erikson, D. Med. Research Council (Brit.) Spec. Rept. Ser. No. **203:** 14–15, 1935).

Morphology: Growth made up of long, straight, profusely branching filaments. Aerial mycelium produces short and straight sporophores. Spores small, round.

Sucrose nitrate agar: Growth moist, cream-colored. Aerial mycelium powdery, white.

Glucose-asparagine agar: Growth cream-colored. Aerial mycelium white.

Nutrient agar: Growth moist, cream-colored. Aerial mycelium powdery, white.

Gelatin: Colonies smooth, shining, floating on liquefied medium. Aerial mycelium powdery, white. Good liquefaction. No soluble pigment.

Milk: Coagulation, slow peptonization. Surface ring pinkish-brown; medium later becomes dark brown.

Starch: No hydrolysis.

Source: Blood culture of a woman with acholuric jaundice. No record concerning actual pathogenicity.

130. *Streptomyces kitasatoensis* Hata *et al.*, 1953 (Hata, T., Koga, F., and Kanamori, H. J. Antibiotics (Japan) **6A:** 109–112, 1953).

Morphology: Sporophores produce primary and secondary verticils. A few spirals were also found. Spores oval or cylindrical, 1.9 to 1.3 by 0.9 μ.

Sucrose nitrate agar: Growth yellow to light yellowish-brown. Some strains form no aerial mycelium even after prolonged cultivation; others form thick grayish-white aerial mycelium. Soluble pigment light yellowish.

Glucose-asparagine agar: Growth brown to dark brown, restricted, with raised center. Aerial mycelium thin, grayish or mouse-grayish. Soluble pigment brown.

Nutrient agar: Growth brown, restricted, with raised center. No aerial mycelium. Soluble pigment brown.

Starch agar: Growth colorless; reverse yellow to yellowish-brown. Aerial mycelium yellowish-white, cottony or flocculent.

Tyrosine agar: Growth brown to dark brown. Aerial mycelium grayish-white, thin, later becoming cottony. Tyrosinase reaction positive.

Potato: Growth yellowish-brown and wrinkled. No aerial mycelium. Color of plug light brown.

Gelatin: Growth dark brown. Soluble pigment dark brown. Liquefaction slow at beginning, but complete liquefaction 4 weeks later.

Milk: No coagulation; slow peptonization. No clearing of milk, but heavy brown precipitate on bottom; color of liquid in upper portion brownish.

Starch: Hydrolysis positive.

Cellulose: No decomposition.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Carbon utilization: Utilizes glucose, starch, dextrin, glycerol, galactose, maltose, sucrose, trehalose, inositol, sorbitol, sodium succinate, sodium citrate, and sodium acetate. Does not utilize xylose, raffinose, rhamnose, lactose, arabinose, mannose, mannitol, inulin, dulcitol, fructose, salicin, or esculin.

Antagonistic properties: Produces an antibiotic, leucomycin.

Remarks: *S. kitasatoensis* is similar to *S. reticuli* in morphology of the mycelium, cultural characteristics, and utilization of carbon sources, but different in several other respects.

131. *Streptomyces kitasawaensis* Harada and Tanaka, 1956 (Harada, Y. and Tanaka, S. J. Antibiotics (Japan) **9A:** 113–117, 1956).

Morphology: Sporophores straight; no spirals.

Sucrose nitrate agar: Growth cream to yellow. Aerial mycelium white with pale pinkish tinge.

Calcium malate agar: Growth cream-colored. Aerial mycelium white. No soluble pigment.

Glucose-asparagine agar: Growth has brownish tinge. Aerial mycelium white with grayish tinge. Soluble pigment pale yellowish-brown to pale greenish-yellow.

Nutrient agar: Growth brownish. Aerial mycelium absent or scarce. Soluble pigment dark brown.

Starch agar: Growth pale grayish-brown to pale blackish-brown. Aerial mycelium white. Soluble pigment pale greenish-yellow to pale yellowish-brown.

Gelatin: Growth white to gray. Aerial mycelium absent or scarce. Soluble pigment dark brown. No or weak liquefaction.

Potato: Growth brown. Aerial mycelium white. Soluble pigment dark brown.

Milk: Growth in form of dark brownish ring. Coagulation and peptonization.

Carbon utilization: Utilizes D(+)xylose, D-mannitol, L-arabinose, salicin. Does not utilize L(+)rhamnose, D-maltose.

Antagonistic properties: Produces an antitumor substance, carzinocidin.

Habitat: Soil.

132. *Streptomyces lanatus* Frommer, 1959 (Frommer, W. Arch. Mikrobiol. **32:** 203, 1959).

Morphology: Sporophores long, straight or wavy, with short side branches; the ends of these are more entangled than spiral-shaped.

Glycerol-sucrose agar: Growth abundant, cottony, with red-brown reverse. Soluble pigment brown to dark red-brown.

Glucose-asparagine agar: Growth rose-brown. Aerial mycelium velvety to cottony, rose to gray-green. Soluble pigment brownish.

Calcium malate agar: Growth colorless to yellowish. Aerial mycelium powdery, gray to gray-green. No soluble pigment.

Nutrient agar: Growth yellow-brown. Aerial mycelium cream-colored or lacking. Soluble pigment yellow-brown to dark brown.

Starch: Weak hydrolysis.

Potato: Growth yellow-brown. Aerial mycelium powdery, white. Soluble pigment black.

Gelatin: Growth yellow. Aerial mycelium yellow. Soluble pigment brown to red-brown. Liquefaction medium.

Milk: Growth abundant, dark brown. Aerial mycelium powdery, cream-colored. Coagulation, slow peptonization.

Cellulose: No growth.

Antagonistic properties: Produces actinomycin.

Remarks: Closely related to *S. purpureochromogenes* and *S. phaeochromogenes.*

133. *Streptomyces lavendulae* (Waksman and Curtis, 1916) Waksman and Henrici,

1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 126, 1916; **8:** 130, 1919).

Morphology: Sporophores long, monopodially branched; short, compact spirals of the dextrorse type, 5 to 8 μ in diameter; spirals sometimes open. Some strains form no spirals, according to Okami (1956). Spores oval, 1.0 to 1.2 by 1.6 to 2.0 μ, smooth (Pl. V, Ea).

Sucrose nitrate agar: Growth thin, spreading, colorless to cream-colored. Aerial mycelium cottony, white, becoming vinaceous-lavender. No soluble pigment.

Glycerol malate agar: Growth cream-colored. Aerial mycelium lavender. No soluble pigment.

Glucose-asparagine agar: Growth yellowish. Aerial mycelium white with lavender tinge. No soluble pigment.

Nutrient agar: Growth wrinkled, gray. No aerial mycelium. Soluble pigment brown.

Starch agar: Growth restricted, glistening, transparent, rose-colored. Aerial mycelium lavender. Good hydrolysis of starch.

Potato: Growth thin, wrinkled, cream to yellowish. No aerial mycelium. Soluble pigment black.

Gelatin: Surface growth creamy to brownish. Aerial mycelium absent or white. Liquefaction slow. Soluble pigment brown.

Milk: Cream-colored ring. No coagulation; good peptonization.

Cellulose: Growth scant.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Temperature: Optimum 37°C.

Antagonistic properties: Various strains of this organism produce antibiotics. One such antibiotic, streptothricin, is active both *in vitro* and *in vivo* against various gram-positive and gram-negative bacteria, fungi, and actinomycetes. Certain other strains produce an antiviral agent, ehrlichin.

Habitat: Soil.

Remarks: Numerous strains and varieties of this organism have been isolated. It is sufficient to mention *S. lavendulae* var. *japonicus*, and several of the cultures listed by Gause *et al.* (1957), notably *A. flavotricini*, *A. toxytricini*, and *A. violascens*. Ettlinger *et al.* (1958) considered *S. acidomyceticus* and *S. virginiae* as members of this group. Krassilnikov (1949) considered this species as a variety of *A. chromogenes*. Okami (1956) and Rangaswami (1958) made a detailed study of numerous representatives of this species or species-group.

Morais *et al.* (1958) described a variety of *S. lavendulae* as *brasilicus*, the aerial mycelium being red-pink or red-brown but not lavender and not utilizing salicin.

Type species: IMRU 3440.

134. *Streptomyces lieskei* (Duché, 1934) Waksman and Henrici, 1948 (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, 1934).

Morphology: Sporophores form oval spores.

Glucose-asparagine agar: Growth cream-colored, later yellowish to green. Aerial mycelium white, later yellowish, growing from the edge toward the center. Soluble pigment dirty yellow to yellow-green.

Nutrient agar: Growth cream-colored. Aerial mycelium white. Soluble pigment yellowish.

Gelatin: Growth cream-colored. Aerial mycelium white. No soluble pigment. Liquefaction rapid.

Milk: Growth cream-colored. No aerial mycelium. Peptonization without coagulation. After 20 days the whole milk becomes a clear yellowish liquid.

Tyrosine medium: Growth rapid. Aerial mycelium whitish-yellow. Soluble pigment yellowish to orange-yellow.

Coagulated serum: Growth colorless. Liquefaction rapid.

Remarks: Related to *S. alboflavus* and *S. albidoflavus*.

135. *Streptomyces limosus* Lindenbein,

1952 (Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Substrate mycelium divides completely into coccoidal pieces. Some similarity to *Nocardia*. Aerial mycelium gray, produced in certain media.

Glycerol nitrate agar: Growth colorless, later becoming deep yellow. No aerial mycelium. Soluble pigment citron-yellow.

Glucose-asparagine agar: Growth lemon-yellow, later becoming black with yellow reverse. Aerial mycelium white, later ash-gray. Soluble pigment lemon-yellow.

Glycerol malate agar: Growth dark yellow. Aerial mycelium white, later ash-gray. Soluble pigment golden yellow.

Nutrient agar: Growth light brown. No aerial mycelium. Soluble pigment light brown. Melanin-positive.

Glucose-peptone agar: Growth yellow-brown. Aerial mycelium ash-gray. Soluble pigment yellow-brown.

Starch-nitrate agar: Growth brownish-yellow. Aerial mycelium gray-white. Soluble pigment light yellow. Hydrolysis strong.

Potato: Growth brownish-yellow. Aerial mycelium gray-white. Soluble pigment lemon-yellow to sulfur-yellow.

Gelatin: Growth yellow-brown. No aerial mycelium. Soluble pigment dark brown. Liquefaction complete.

Milk: Growth lichenoid, light yellow. Aerial mycelium gray-white. Soluble pigment light brown. Strong peptonization.

Cellulose: No growth.

Antagonistic properties: None.

Source: Isolated from the slime of a river bank.

Remarks: Related to *S. flavovirens*.

136. *Streptomyces lipmanii* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 123, 1916; **3:** 121, 1919).

Morphology: Sporophores straight, no spirals. Spores oval, 0.8 to 1.1 by 1.0 to 1.5 μ.

Sucrose nitrate agar: Growth abundant, raised, colorless, becoming light brown and wrinkled. Aerial mycelium white, turning gray to dark gray. No soluble pigment.

Glycerol malate agar: Growth colorless, becoming dark brown. Aerial mycelium mouse-gray. No soluble pigment.

Glucose-asparagine agar: Growth spreading, light yellow. No aerial mycelium. No soluble pigment.

Nutrient agar: Growth wrinkled, glossy, yellow. No aerial mycelium. No soluble pigment.

Potato: Growth abundant, wrinkled, cream-colored. Aerial mycelium white to gray. Soluble pigment purplish.

Gelatin: Cream-colored, flaky sediment. Aerial mycelium white-gray. Liquefaction medium to rapid. Melanin-negative.

Milk: Cream-colored ring. Coagulation and peptonization.

Starch media: Growth transparent, becoming dark with age. No aerial mycelium. Hydrolysis medium.

Cellulose: No or very scant growth.

Invertase: Positive.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 25°C.

Antagonistic properties: Good, though some strains show no activity.

Habitat: Soil.

Remarks: Ettlinger *et al.* (1958) consider this organism as a strain of *S. griseus*. Tresner and Danga reported that their strain produced a grayish-yellow-buff aerial mycelium. Krassilnikov (1949) considered it as a variety of *A. viridis*.

Type culture: IMRU 3331.

137. *Streptomyces loidensis* (Millard and Burr, 1926) Waksman (Millard, W. A., and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight and spiral-forming. Spores cylindrical to spherical, 0.9 to 1.0 by 0.9 to 0.95 μ.

Sucrose nitrate agar: Growth thin, flat, gray to yellowish-olive. Aerial mycelium scant, olive-colored. Soluble pigment yellow.

Nutrient potato agar: Growth good, gray. Aerial mycelium olive-buff. Soluble pigment golden brown.

Gelatin: Growth gray. Aerial mycelium scant, white. Liquefaction rapid. Soluble pigment yellow.

Milk: Surface growth excellent. Aerial mycelium white. Coagulation and rapid peptonization.

Starch: Hydrolysis.

Nitrate reduction: None.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab.

138. *Streptomyces longisporoflavus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 30, 1941).

Morphology: Sporophores produce long open spirals. Spores cylindrical or elongated, 1.0 to 1.5 by 0.7 μ, some rounded at ends and swollen in center.

Agar media: Growth yellow to lemon-yellow or dirty yellow, seldom golden yellow. Pigment insoluble. Aerial mycelium well developed, velvety, whitish-yellow to brownish-yellow.

Gelatin: Liquefaction medium.

Milk: Coagulation and slow peptonization.

Starch: Hydrolysis weak.

Cellulose: No growth.

Nitrate reduction: Positive.

Sucrose: No inversion.

Antagonistic properties: Weakly antagonistic.

Remarks: Some strains, such as *S. flavoviridis*, sometimes have a greenish or greenish-yellow color instead of a yellow color. This organism and related forms belong to the same group as *S. griseoflavus* and *S. microflavus*. The form described by Gause *et al.* (1957) as *A. aurini* also belongs to this group.

139. *Streptomyces lucensis* Arcamone *et al.*, 1957* (Arcamone, F., Bertazzoli, C., Canevazzi, G., DiMarco, A., Ghione, M., and Grein, A. Giorn. Microbiol. **4**: 119–128, 1957).

Morphology: Aerial hyphae long, branched, and hooked at the tip. Spirals produced abundantly.

Glycerol-glycine agar: Growth abundant, lemon-yellow. Aerial mycelium gray-brown. Some soluble pigment produced.

Glucose-asparagine agar: Growth abundant. Aerial mycelium hazel-brown; scanty clusters of white, short, sterile hyphae. No soluble pigment.

Potato-glucose agar: Growth abundant, smooth, yellowish. Aerial mycelium abundant, buff-gray to hazel-brown. Soluble pigment ash-gray, later turning gray-brown.

Yeast-glucose agar: Growth dark brown. Aerial mycelium whitish. Soluble pigment dark brown.

Starch agar: Growth abundant, colorless to yellowish-brown. Aerial mycelium powdery, buff-gray to light brown. No soluble pigment. Moderate starch hydrolysis.

Oatmeal agar: Growth yellowish and smooth. Aerial mycelium hazel-brown. No soluble pigment.

Potato plug: Growth abundant, wrinkled. Aerial mycelium light gray to hazel-brown. Plug surface turns dark brown around culture.

Gelatin: Growth abundant, brown. Aerial mycelium white, turning grayish-brown. Substrate is strongly darkened within 3 days. No liquefaction.

Antagonistic properties: Produces an antifungal antibiotic, etruscomycin, of the tetraene type.

Type culture: IMRU 3783.

140. *Streptomyces luridus* (Krassilnikov *et al.*, 1957) Waksman (Krassilnikov, N. A., Koreniako, A. I., Meksina, M. M., Vale-

*Supplemented by personal communication.

dinskaia, L. K., and Vesselov, N. M. Mikrobiologiya **26:** 558–564, 1957).

Morphology: Substrate mycelium monopodially branched, 0.7 to 0.8 μ in diameter. Sporophores produce spirals with 1 to 3 turns. Spores spherical, oval, seldom elongated. Sporulation is generally weak, occurring only on certain media; spiral formation occurs seldom, largely on synthetic media with a limited amount of sucrose, and on starch media.

Sucrose nitrate agar: Growth yellow-orange. Aerial mycelium white with rose tinge.

Nutrient agar: Substrate growth colorless, free of aerial mycelium. No soluble pigment. In old cultures, clumps of aerial hyphae may be formed.

Potato agar: Growth yellow-orange. Aerial mycelium white with rose tinge. Crystals of salts deposited along the mycelium in the substrate.

Milk: Coagulation weak; rapid peptonization.

Gelatin: Not liquefied in 10 days.

Starch: Moderate hydrolysis.

Nitrate: Reduced.

Sucrose: Not inverted.

Cellulose: No growth.

Carbon utilization with acid formation: arabinose, inositol, sorbitol; no acid from glucose, lactose, rhamnose, xylose, inulin, inositol, mannitol, or dulcitol.

Antagonistic properties: Produces antibacterial and antiviral (luridin) substances.

Remarks: Cannot be distinguished from *S. fradiae* in its morphological and cultural properties, but is different in its biochemical and its antibiotic activities.

141. *Streptomyces luteoverticillatus* Shinobu, 1956 (Shinobu, R. Mem. Osaka Univ. B (N.S.) **5:** 84–93, 1956).

Morphology: Primary and secondary verticils produced on various synthetic media. Spores coccoid to elliptical, about 0.8 μ long.

Sucrose nitrate agar: Growth pale brown. Aerial mycelium cottony, brownish-white to brown.

Glucose-asparagine agar: Growth thin, brown. Aerial mycelium cottony, yellow-white. Soluble pigment pale brown.

Nutrient agar: Growth excellent, deep brown. Aerial mycelium white to yellow to green. Soluble pigment deep brown.

Potato plug: Growth heavy, brown. Aerial mycelium yellow to greenish. Soluble pigment brown.

Milk: Aerial mycelium heavy, brown. Coagulation uncertain; peptonization strong. Soluble pigment brown.

Gelatin: Strong liquefaction.

Diastase reaction: Strong.

Tyrosinase reaction: Strong.

Carbon utilization: Fructose, mannitol, and inositol utilized. Xylose, rhamnose, sucrose, lactose, and raffinose not utilized.

Habitat: Soil.

Remarks: Resembles *S. verticillatus*.

142. *Streptomyces lydicus* DeBoer *et al.*, 1955 (DeBoer, C., Dietz, A., Silver, W. S., and Savage, G. M. Antibiotics Ann. 1955–1956, p. 886–892).

Morphology: Sporophores long, slightly coiled at tip. Spores spherical to oval.

Sucrose nitrate agar: Substrate growth buff-colored. Aerial mycelium white.

Nutrient agar: Some substrate growth. No aerial mycelium. Soluble pigment yellowish.

Casein-yeast extract-beef agar: Growth olive-tan. Aerial mycelium gray-white with flecks of black. Soluble pigment olive-tan.

Glucose-peptone agar: Aerial mycelium gray-white. Soluble pigment yellow.

Starch agar: Growth good. Aerial mycelium pink-gray-white. Hydrolysis good to excellent.

Gelatin: Some growth. No aerial mycelium. Liquefaction positive. Soluble pigment olive-colored.

Milk: Ring around surface. Peptonization positive.

Nitrate reduction: Positive.

Carbon utilization: Most sugars and organic acids utilized, but not rhamnose, inulin, dulcitol, inositol, or the sodium salts of formic, oxalic, tartaric, and salicylic acids.

Production of H_2S: Negative.

Antagonistic properties: Produces an antibiotic, streptolydigin, active against various bacteria.

Remarks: Related to *S. diastaticus*.

143. *Streptomyces macrosporeus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 346, 1958).

Morphology: Sporophores monopodially branched along the whole axis with open, irregular spirals. Spores large, 1.7 to 2 by 1.5 to 2 μ; short spines (Plate II m).

Glycerol nitrate agar: Substrate growth yellow. Aerial mycelium white-yellow to ash-gray. Soluble pigment golden yellow.

Glucose-asparagine agar: Growth milk-white. No aerial mycelium. Soluble pigment whitish-yellow.

Calcium malate agar: Growth yellow. Aerial mycelium white-yellow to ash-gray. Soluble pigment white-yellow.

Starch agar: Growth light yellow. Aerial mycelium white-gray. Good hydrolysis of starch.

Potato: Growth abundant, light yellow to golden yellow. Aerial mycelium ash-gray.

Gelatin: Growth sparse. Liquefaction slow. No soluble pigment.

Milk: Pellicle light yellow to yellow-brown. Aerial mycelium white to white-gray. Coagulation strong; no peptonization.

Antagonistic properties: Produces an antibiotic, carbomycin.

Habitat: Soil in Madras, India.

144. *Streptomyces maculatus* (Millard and Burr, 1926) Waksman and Henrici, 1948 (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Growth tough, shiny, cartilaginous. Aerial mycelium rarely produced, though in certain strains it may frequently occur, especially when grown on organic media. Sporophores straight, short. Spores spherical, 0.5 to 0.6 μ.

Sucrose nitrate agar: Growth orange-yellow to orange-red; as the culture ages it may change to dark green or black. No aerial mycelium.

Nutrient potato agar: Growth vinaceous-tawny. Soluble pigment vinaceous-tawny.

Potato: Growth restricted, raised, pinkish. Aerial mycelium scant, white. Soluble pigment gray to brown.

Gelatin: Growth limited. Liquefaction slow.

Milk: Growth slight. No coagulation; no peptonization.

Starch: Hydrolyzed.

Nitrate reduction: Negative.

Tyrosinase reaction: Negative.

Oxygen requirement: Said to grow well under anaerobic conditions.

Paraffin: Not utilized.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab and soil.

Type culture: IMRU 3376.

145. *Streptomyces madurae* (Vincent, 1894) nov. comb. (Vincent, H. Ann. inst. Pasteur **8:** 129, 1894).

Synonyms: *N. babiensis*, *N. brumpti*, *N. madurae*, and *N. salmonicolor* (*A. salmonicolor* Millard and Burr, 1926). Baldacci (1944) listed 17 synonyms.

Strains of this organism were reported by various investigators, most recently by Gonzalez Ochoa and Sandoval (1951), to form, under certain conditions of culture and on certain media, such as grain, an aerial mycelium, with straight or spiral-shaped sporophores; the spores were cylindrical or oval. This led them to consider this organism as a *Streptomyces*. Mariat (1957) was also of the same opinion. MacKinnon and Artagaveytia-Allende (1956) consider

the generic position of this species as far from settled.

Morphology: Growth red to red-brown or pink. In tissues it forms granules consisting of radiating actinomycosis. Initial branched mycelium is said to be nonsegmented. Not acid-fast. Aerial mycelium white and pink in color.

Glucose-asparagine agar: Growth cream-colored. Some cultures give reddish pigmentation.

Protein media: Growth good, pinkish. Soluble pigment brown.

Gelatin: Growth glistening, at first white, then buff to rose or crimson. Soluble pigment irregular and unpredictable, occasionally red. Gelatin slowly liquefied.

Milk: No change, or slight; coagulation slow, if any; peptonization slow.

Carbon utilization: Utilizes starch, glucose, mannitol, and xylose, but not lactose or paraffin.

Nitrate reduction: Positive.

Pathogenicity: This property was variously reported. Topley and Wilson (1946) stated that this organism causes a local tissue disease when inoculated under the skin in guinea pigs. Often reported as not pathogenic for the usual laboratory animals; pathogenic for monkeys.

Source: Wide geographical distribution. Madura foot and other substrates.

146. *Streptomyces marginatus* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight. Spores oval to spherical, 0.9 by 0.8 μ.

Sucrose nitrate agar: Growth thin, echinate. Aerial mycelium olive-buff. Soluble pigment cream-colored.

Nutrient potato agar: Growth heavy, gray. Aerial mycelium white to whitish-yellow. Soluble pigment light golden brown to deep golden brown.

Potato: Growth good, raised. Aerial mycelium abundant, buff to olive-buff. Plug at first gray, later becoming black.

Gelatin: Growth thin, pale olive-gray. Aerial mycelium abundant, pale gray to olive-buff. Soluble pigment buff. Liquefaction rapid.

Milk: Growth good. Aerial mycelium white. Coagulation and peptonization.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab.

147. *Streptomyces marinolimosus* (Zobell and Upham, 1944) Waksman (ZoBell, C. E. and Upham, H. C. Bull. Scripps Inst. Oceanogr. Univ. Calif. **5:** 239–292, 1944).

Morphology: Aerial mycelium consists of branching filaments with chains of spores. Spores 0.9 μ in diameter.

Agar media: Growth dull. Aerial mycelium white to pinkish, powdery, rough, in concentric circles. Odor of freshly turned soil.

Potato: Growth yellow, becoming white, powdery, raised, rugose. Potato darkened.

Gelatin: Growth in form of flat, yellowish, circular colonies, with tendency to grow together. Liquefaction crateriform. Melanin-negative.

Sea water broth: Growth in form of light yellow clumps; pellicle produced on surface. Earthy odor.

Milk: Growth in form of pellicle. Complete peptonization in 20 days.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Source: Marine mud.

Remarks: All differential media were prepared with sea water.

148. *Streptomyces marinus* (Humm and Shepard, 1946) nov. comb. (Humm, H. J. and Shepard, K. S. Duke Univ. Marine Sta. Bull. **3:** 77, 1946).

Morphology: Sporophores sometimes form

loose spirals. Spores spherical to oval, 0.8 to 1.2 μ, produced as a dark gray area in center of colonies.

Agar media: Growth sparingly branched, dense, entangled, frequently forming concentric rings in response to alternate periods of light and darkness. Aerial mycelium white. Spores gray to dark gray. No soluble pigment.

Gelatin: Growth arborescent. Liquefaction stratiform, slow. Melanin-negative.

Milk: Peptonization complete, usually within 1 month.

Starch: Vigorous hydrolysis.

Cellulose: Not attacked.

Chitin and alginic acid: Attacked.

Agar: Slowly digested, softened, not liquefied. Growth on agar in culture dish surrounded by rather wide, gently sloping depression. Gelase field relatively wide, with distinct margin. Irish moss and Hypnea gels also slowly digested.

Nitrate reduction: Usually negative. In some media, slight nitrite is produced after 10 days' incubation, especially if glucose is present.

Production of H_2S: Positive.

Indole: Not formed.

Carbon utilization: Acid produced from galactose, glucose, fructose, mannose, cellobiose, lactose, maltose, sucrose, and glycerol. Arabinose, xylose, rhamnose, and sorbitol utilized without acid production. No growth with raffinose, salicin, inulin, dulcitol, inositol, ethyl alcohol, or ethylene glycol. Utilizes acetic, citric, lactic, propionic, succinic, and *iso*-valeric acids. Does not utilize butyric, gluconic, maleic, malonic, and oxalic acids.

Habitat: Marine sediments.

149. *Streptomyces mashuensis* Sawazaki *et al.*, 1955 (Sawazaki, T., Susuki, S., Nakamura, G., Kawasaki, M., Yamashita, S., Isono, K., Anzai, K., Serizawa, Y., and Sekiyama, Y. J. Antibiotics (Japan) **8A:** 44–47, 1955).

Morphology: Sporophores straight, no spirals; numerous primary and secondary verticils.

Sucrose nitrate agar: Growth yellow; reverse yellow-green. Aerial mycelium abundant, powdery.

Glucose-asparagine agar: Growth powdery, grayish-white, reverse yellow-brown. No aerial mycelium.

Nutrient agar: Growth cream-colored; reverse brown. No aerial mycelium. No soluble pigment.

Starch agar: Growth marguerite-colored; margin cottony, primrose-pink; reverse yellow-brown, margin white. Aerial mycelium white. Strongly diastatic.

Potato: Growth spreading, dark cream-colored. Aerial mycelium limited. Limited discoloration of plug.

Gelatin: Growth white. No aerial mycelium. Soluble pigment pinkish. Liquefaction medium.

Nitrate reduction: Negative.

Carbon utilization: Xylose, glucose, sucrose, trehalose utilized. Rhamnose, raffinose, salicin, mannitol, lactose, arabinose not utilized.

Antagonistic properties: Produces two antibiotics, streptomycin and a labile substance active against mycobacteria, fungi, and *B. subtilis*.

Remarks: Okami *et al.* (1959b) made a detailed study of this organism. They reported, instead of the yellow growth on synthetic media, poor colorless growth.

150. *Streptomyces matensis* Margalith *et al.*, 1959 (Margalith, P., Beretta, G., and Timbal, M. T. Antibiotics & Chemotherapy **9:** 71–75, 1959).

Morphology: Sporophores produce verticils, the branches forming spirals. Spores spherical.

Sucrose nitrate agar: Growth colorless, the reverse being hyaline to light violet-gray. Aerial mycelium powdery, gray. Faint bluish pigment.

Glucose-asparagine agar: Growth hyaline with pinkish reverse. Aerial mycelium present. No soluble pigment.

Calcium malate agar: Growth poor. No aerial mycelium.

Nutrient agar: Growth abundant, colorless. Aerial mycelium whitish. Soluble pigment amber.

Starch: Strong hydrolysis.

Potato: Growth abundant. Aerial mycelium light gray. No soluble pigment.

Gelatin: Partial liquefaction. No soluble pigment.

Milk: No coagulation; some peptonization.

Nitrate reduction: Negative.

Cellulose: Good growth.

Carbon utilization: Utilizes glucose, sucrose, lactose, galactose, rhamnose, xylose, inositol, sodium succinate, and others. Does not utilize sucrose, raffinose, glycine, or sodium citrate.

Antagonistic properties: Produces an antibacterial agent, matamycin.

Habitat: Soil.

Remarks: Related to *S. noboritoensis* and *S. spiralis.*

Characteristic properties: Culture said to be melanin-positive; it produces gray to violet spore masses.

151. *Streptomyces mediocidicus* Okami *et al.*, 1954 (Okami, Y., Utahara, R., Nakamura, S., and Umezawa, H. J. Antibiotics (Japan) **7A:** 98–103, 1954).

Morphology: Aerial mycelium sometimes produces verticils, depending on composition of medium; no spirals.

Glycerol nitrate agar: Growth colorless to yellowish. Aerial mycelium absent, or white patches. Soluble pigment absent, or slightly yellowish brown.

Glucose-asparagine agar: Same as above.

Starch agar: Same as above. Diastatic action weak or medium.

Nutrient agar: Growth colorless or slightly yellowish. No aerial mycelium. Soluble pigment brownish. Melanin-positive.

Potato: Growth yellowish or light yellowish-brown. Aerial mycelium absent or white. No soluble pigment.

Gelatin: Growth yellowish-brown. No aerial mycelium. Soluble pigment brown. Strong liquefaction.

Blood agar: Growth yellowish-brown to reddish-brown. Aerial mycelium absent or white. Hemolytic action strong.

Milk: Surface ring colorless to yellowish. No aerial mycelium. Soluble pigment slightly brownish. Slow coagulation and peptonization.

Nitrate: No reduction.

Antagonistic properties: Produces an antifungal substance, mediocidin, a polyene of the hexaene type.

Type culture: IMRU 3777.

152. *Streptomyces melanocyclus* (Merker, 1911, *emend.* Krainsky) Waksman and Henrici, 1948 (Merker, E. Centr. Bakteriol. Parasitenk. Abt. II, **31:** 589, 1912; Krainsky, A. *ibid.* **41:** 649–688, 1914).

Morphology: Spores spherical, 0.9 μ.

Agar media: Growth much folded, red. Aerial mycelium dark brown. Soluble pigment dark brown, turns whole culture red-brown to almost black with a shade of red.

Calcium malate agar: Colonies small, flat, orange-red. Aerial mycelium black, occurring along the edges.

Gelatin: Growth poor. Liquefaction rapid.

Milk: Coagulation and rapid peptonization.

Starch: Hydrolysis.

Cellulose: Good decomposition; black circles produced on paper.

Nitrate reduction: Positive.

Sucrose: Inverted.

Pigment: Insoluble in water and in organic solvents. Considered by Kriss to be related to the melanins.

Antagonistic properties: Strong effect

upon various bacteria; some strains show no activity.

Habitat: Soil.

Remarks: *A. melanosporeus* (Krainsky, 1914) and *A. melanogenes* (Rubentschik 1928) are related to above species.

153. *Streptomyces melanogenes* Sugawara and Onuma, 1957 (Sugawara, R. and Onuma, M. J. Antibiotics (Japan) **10A:** 138–142, 1957).

Morphology: Sporophores monopodially branched; no spirals, sometimes slight curvature. Spores cylindrical, 1.7 to 0.8 by 0.9 to 0.5 μ.

Sucrose nitrate agar: Growth moist, folding, colorless to grayish-red-brown; reverse yellow-orange. Aerial mycelium thin, brownish-white. Soluble pigment brownish-yellow.

Glucose-asparagine agar: Growth colorless to cream-colored with dark reddish center; reverse dark yellow-orange. Aerial mycelium pale grayish-white. Soluble pigment yellowish-brown.

Calcium malate agar: Growth colorless to brownish-yellow to grayish-blue-black. Aerial mycelium yellow-white. Soluble pigment greenish-yellow to brown.

Nutrient agar: Growth cream-colored to brown. No aerial mycelium. Soluble pigment reddish-brown.

Potato: Growth folded, colorless to yellowish-brown. Aerial mycelium brownish or grayish-white. Soluble pigment dark yellowish-brown.

Gelatin: Growth colorless to dark brown. Aerial mycelium white to gray. Soluble pigment pale yellowish-brown. Liquefaction weak.

Milk: Cream-colored to dark brown ring. Soluble pigment pinkish-brown.

Blood agar: Growth glistening, yellowish-gray to dark olive-gray. No aerial mycelium. Soluble pigment dark brown. Hemolysis positive.

Antagonistic properties: Produces a melanin-like tumor-inhibiting substance.

Remarks: Resembles *S. phaeochromogenes*, *S. griseocarneus*, and *S. cinnamonensis*.

154. *Streptomyces michiganensis* Corbaz *et al.*, 1957 (Corbaz, R., Ettlinger, L., Keller-Schierlein, W., and Zähner, H. Arch. Mikrobiol. **26:** 192–208, 1957).

Morphology: Sporophores straight, arranged in sympodially branched clusters; no spirals. Spores smooth (Pl. II i).

Glycerol nitrate agar: Growth whitish-yellow. Aerial mycelium velvety, white to yellowish to greenish-gray.

Calcium malate agar: Growth thin, golden yellow. Aerial mycelium chalk-white, becoming light yellow.

Glucose-asparagine agar: Growth thin, white to yellow, changing to light yellow-red. Aerial mycelium velvety, white-yellow.

Glucose-peptone agar: Growth wrinkled, at first light brown, then copper-red, finally reddish-brown. Aerial mycelium velvety, greenish-gray. Soluble pigment reddish-brown.

Gelatin: Pellicle light brown. Aerial mycelium powdery, chalk-white. Liquefaction slow. Soluble pigment brown.

Starch: No hydrolysis.

Potato: Growth light yellow. Aerial mycelium velvety, white-gray to white-yellow. Soluble pigment gray-black.

Milk: Pellicle light brown. Aerial mycelium sparse. Coagulation and peptonization.

Tyrosinase reaction: Positive.

Antagonistic properties: Produces actinomycin X.

Carbon utilization: Xylose, arabinose, fructose, galactose, maltose, mannitol, salicin utilized. Rhamnose, sucrose, lactose, raffinose, inulin not utilized.

Habitat: Soil.

155. *Streptomyces microflavus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 686, 1914).

Morphology: Spores spherical to rod-shaped, often produced in pairs or in chains, 2.0 by 2 to 5 μ.

Calcium malate agar: Colonies minute, yellow. No aerial mycelium.

Glucose-asparagine agar: Aerial mycelium produced late (12 days), rose-yellow.

Nutrient agar: Colonies yellow. Aerial mycelium produced late, yellowish-rose.

Potato: Growth yellowish, slimy mass. No aerial mycelium. Melanin-negative.

Gelatin: Colonies small, yellowish. Liquefaction rapid.

Milk: Rapid coagulation and peptonization.

Invertase: Negative.

Starch: Diastatic action strong.

Cellulose: Growth scant, white.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Antagonistic properties: Said to produce a form of streptothricin.

Habitat: Soil.

Remarks: According to Ettlinger *et al.* (1958), this organism belongs to the *S. griseus* series.

Type culture: IMRU 3332; ATCC 13,231.

156. *Streptomyces mirabilis* Ruschmann, 1952 (Ruschmann, G. Pharmazie **7:** 542–550, 639–648, 823–831, 1952).

Morphology: Sporophores straight, without spirals or curvature.

Agar media: Aerial mycelium white, cottony.

Nutrient agar: Growth poor, forming slimy surface. No aerial mycelium.

Glucose agar: Growth grayish-brown. No aerial mycelium. Soluble pigment brown.

Potato: Growth good, lichenoid. Soluble pigment dark brown to black.

Gelatin: Good flaky growth. Rapid liquefaction. Soluble pigment dark brown to black.

Milk: Surface growth covered with white, fluffy aerial mycelium. Coagulation and peptonization positive. Liquefied portion colored black.

Fats: Ready utilization.

Temperature: Optimum 29°C. No growth at 37°C.

Antagonistic properties: Antagonistic effect strongest in freshly isolated cultures. Property lost on cultivation; activity lost first against gram-negative, rod-shaped bacteria, cocci remaining most sensitive. Produces antibiotic miramycin.

Remarks: Highly proteolytic and lipolytic. Grows best on complex organic media, at slightly acid reaction, pH 6.0 to 6.6.

157. *Streptomyces mitakaensis* Arai *et al.*, 1958 (Arai, M., Karasawa, K., Nakamura, S., Yonehara, H., and Umezawa, H. J. Antibiotics (Japan) **11A:** 14–20, 1958).

Morphology: Sporophores short, branched; spirals produced. Spores spherical, 1.2 to 1.5 μ.

Sucrose nitrate agar: Growth good, colorless or white to dark yellowish-brown. Aerial mycelium powdery, abundant, light gull-gray. No soluble pigment.

Glucose-asparagine agar: Growth good, white to brown; reverse becomes dark brown. Aerial mycelium powdery, abundant, gray. No soluble pigment.

Glycerol citrate agar: Growth good, colorless or white to brownish-white; later brownish-yellow. Aerial mycelium powdery, abundant, whitish-gray in center, gray in the edges. No soluble pigment.

Nutrient agar: Growth good, colorless to pale yellowish-brown. Aerial mycelium powdery, abundant, white, with or without gray parts. No soluble pigment.

Starch agar: Growth good, colorless to pale yellowish-brown. Aerial mycelium powdery, abundant, light gray in center, gray at the edges. No soluble pigment. Hydrolysis strong.

Potato plug: Growth good, wrinkled, cream-buff. Aerial mycelium poorly devel-

oped, white or grayish-white. No soluble pigment.

Gelatin: Growth colorless. Aerial mycelium pale gull-gray. Soluble pigment absent, yellow. Liquefaction moderate.

Milk: Growth good, colorless to white. Peptonization and coagulation rapid.

Nitrate reduction: Negative.

Antagonistic properties: Produces an antibiotic, mikamycin, active against gram-positive and acid-fast bacteria.

158. *Streptomyces murinus* Frommer (Frommer, W. Arch. Mikrobiol. **32:** 198, 1959).

Morphology: Sporophores small, monopodially branched, tree-like; spirals compact, with 1 to 3 turns.

Glycerol nitrate agar: Growth greenish-yellow. Aerial mycelium thin, white. Soluble pigment greenish-yellow.

Glycerol-glycine agar: Growth yellow to brown-yellow. Aerial mycelium white-gray to gray-brown. Soluble pigment golden yellow.

Glucose-asparagine agar: Growth yellow, occasionally brown-violet. Aerial mycelium powdery white to gray-white. Soluble pigment yellow to greenish-yellow.

Calcium malate agar: Growth colorless. Aerial mycelium white. No soluble pigment.

Nutrient agar: Growth yellow to greenish-yellow. Aerial mycelium lacking or white. Soluble pigment lacking or golden yellow.

Starch media: Growth colorless to yellowish. Aerial mycelium gray-brown. No hydrolysis after 10 days.

Potato: Growth abundant, golden brown. Aerial mycelium cream-colored to yellow. Soluble pigment questionable.

Gelatin: Growth abundant, golden yellow. Aerial mycelium cream-colored to gray. Soluble pigment yellow to golden yellow. Liquefaction limited. Melanin-negative.

Milk: Growth abundant, golden yellow to yellow-brown. Aerial mycelium powdery, cream-colored. Questionable coagulation and liquefaction.

Cellulose: Growth weak. Aerial mycelium gray-brown. Soluble pigment yellowish.

Antagonistic properties: Produces actinomycin.

159. *Streptomyces naganishi* Yamaguchi and Saburi, 1955 (Yamaguchi, T. and Saburi, Y. J. Gen. Appl. Microbiol. **1:** 201–235, 1955).

Morphology: Sporophores straight with many compact spirals and a few open spirals; spores oval to short rods, 0.8 to 1.4 by 0.5 to 0.7 μ.

Sucrose nitrate agar: Growth colorless, thin. Aerial mycelium powdery, at first white, later colored buff. No soluble pigment.

Calcium malate agar: Growth is at first pinkish-white to pinkish-gray, later becoming whitish-brown. Aerial mycelium whitish. Soluble pigment light pink, but soon disappears.

Nutrient agar: Growth at first colorless to dark cream, later becoming yellowish-brown to brown. No aerial mycelium. Soluble pigment light brown. Melanin-negative.

Starch agar: Growth colorless to creamy with reddish-purple portion. Aerial mycelium abundant, white or smoke-gray to light drab. Soluble pigment absent or faint pink. Good hydrolysis.

Potato: Growth vigorous, at first yellowish-gray. Aerial mycelium white to grayish-white. Soluble pigment deep purple to black.

Gelatin: Growth dark brown with some tint of olive. Soluble pigment deep brown and a more diffusible yellowish-green. Liquefaction moderate.

Milk: Growth vigorous, yellowish-brown, with white aerial mycelium along the glass. Soluble pigment light brown, sometimes reddish-brown. Coagulation and peptonization.

Carbon utilization: Utilizes D-xylose, L-arabinose, L-rhamnose, D-galactose, lactose, raffinose, mannitol, inositol, salicin, acetate, citrate, and succinate; does not utilize sucrose, inulin, sorbitol, or cellulose.

Antagonistic properties: Active against gram-positive and acid-fast bacteria, fungi, and trichomonads.

Remarks: Related to *S. antimycoticus.*

160. *Streptomyces narbonensis* Corbaz *et al.*, 1955 (Corbaz, R., Ettlinger, L., Gäumann, E., Keller, W., Kradolfer, F., Kyburz, E., Neipp, L., Prelog, V., Reusser, R., and Zähner, H. Helv. Chim. Acta **38:** 935–942, 1955).

Morphology: Sporophores straight; no spirals. Spores smooth, cylindrical, 0.8 to 1.1 by 0.7 to 0.9 μ.

Glycerol nitrate agar: Growth thin, colorless to yellowish-brown. Aerial mycelium velvety, whitish-gray. No soluble pigment.

Glucose-asparagine agar: Growth thin, at first colorless, then yellowish-brown. Aerial mycelium sparse, chalk-white. No soluble pigment.

Calcium malate agar: Growth colorless.

FIGURE 43. Hyphae of isolate AA 877, resembling *S. netropsis*, showing character of verticils of sporogenous branches (Reproduced from: Duggar, B. M. *et al.* Ann. N. Y. Acad. Sci. **60:** 85, 1954).

Aerial mycelium white-gray. No soluble pigment.

Glucose-peptone agar: Growth light yellowish, punctiform. Aerial mycelium produced late, powdery, gray-white. No soluble pigment.

Nutrient agar: Growth punctiform, yellowish. No aerial mycelium. No soluble pigment. Melanin-negative.

Starch agar: Growth thin, colorless to yellowish. Aerial mycelium powdery, white. No soluble pigment. Hydrolysis good.

Gelatin: Growth yellowish-white. Aerial mycelium snow-white. Soluble pigment light reddish-brown. Liquefaction slow.

Potato: Growth lichenoid, bluish-gray to reddish-gray. No aerial mycelium. Soluble pigment dark brown.

Milk: Surface ring whitish-yellow. Peptonization without coagulation.

Production of H_2S: Positive.

Antagonistic properties: Produces basic antibiotic, narbomycin, related to picromycin.

Carbon utilization: Utilizes xylose, arabinose, rhamnose, fructose, galactose, saccharose, maltose, raffinose, inulin, salicin, sodium acetate. Does not utilize mannitol, sorbitol, dulcitol, mesoinositol.

Habitat: Soil.

Remarks: Ettlinger *et al.* (1958) consider this organism as belonging to *S. olivaceus.*

161. *Streptomyces netropsis* Finlay and Sobin, 1952 (Finlay, A. C. and Sobin, B. A. U. S. 2,586,762, 1952).

Morphology: Sporophores in form of verticils or terminal clusters on tips of short hyphae (Fig. 43). Spores short, cylindrical, 0.7 by 1.3 μ, smooth (Pl. I a).

Sucrose nitrate agar: Growth thin, pale olive-buff. Aerial mycelium pale vinaceous-fawn. No soluble pigment.

Glucose-asparagine agar: Growth moderate, wrinkled. Aerial mycelium white. Soluble pigment brown.

Calcium malate agar: Growth moderate,

cream to buff. Aerial mycelium white. No soluble pigment.

Nutrient agar: Growth moderate to good, light brown. Aerial mycelium white. Soluble pigment light brown.

Starch agar: Growth moderate, thin; pale olive-buff reverse. Aerial mycelium white. No soluble pigment. Strong hydrolysis.

Potato: Growth poor, waxy, wrinkled, brown. No aerial mycelium. Soluble pigment dark brown.

Gelatin: Moderate surface growth. Aerial mycelium white. Soluble pigment dark brown. No liquefaction.

Milk: Growth poor. No peptonization.

Nitrate reduction: Negative.

Production of H_2S: Variable.

Antagonistic properties: Produces a basic antibiotic, netropsin.

Remarks: Ettlinger *et al.* (1958) report this organism to be melanin-negative. They also consider *S. cinnamomeus* as closely related.

162. *Streptomyces niger* (Rossi-Doria, 1891; *emend.* Krassilnikov, 1949) Waksman (Rossi-Doria, E. Ann. igiene, **1:** 399-438, 1891; Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 53, 1941).

Morphology: Substrate growth of soft consistency. Aerial mycelium produced only on potato and synthetic agar. Sporophores formed only seldom; open spirals, with 3 to 5 turns. Spores oval.

Synthetic agar: Growth black. Aerial mycelium dark gray. No soluble pigment.

Nutrient agar: Growth black. Soluble pigment brown.

Gelatin: Slow liquefaction, in 30 days. Melanin-negative (?).

Milk: No change.

Starch: No growth.

Cellulose: No growth.

Nitrate reduction: Negative.

Sucrose: No inversion.

Temperature: Optimum 25–30°C.

Antagonistic properties: None.

Remarks: This is a very unstable species which dies out rapidly. It easily mutates, giving rise to colorless cultures, producing no aerial mycelium. It appears to be a transition form, if not a true *Nocardia*. *A. niger aromaticus* Berestnew and *A. nigrificans* (Krüger) Wollenweber are listed by Krassilnikov as varieties of *A. niger*. In view of the formation of a soluble brown substance on certain protein media, this organism may belong to one of the chromogenic groups.

163. *Streptomyces nigrifaciens* Waksman, 1919 (Waksman, S. A. Soil Sci. **8:** 167–168, 1919).

Morphology: Sporophores branching with tendency to curl; no true spirals. Spores oval-shaped to elliptical.

Sucrose nitrate agar: Growth colorless. Aerial mycelium thin, gray. No soluble pigment.

Glucose-asparagine agar: Growth cream-colored. Aerial mycelium mouse-gray with white patches.

Nutrient agar: Growth thin, cream-colored. Aerial mycelium gray. Soluble pigment brown.

Starch agar: Growth cream-colored to yellow. Aerial mycelium light buff-gray. Hydrolysis imperfect.

Egg media: Growth abundant, dark brown. No aerial mycelium. Purplish zone around growth.

Potato: Growth gray becoming dark. Aerial mycelium white, appearing late. Soluble pigment black.

Gelatin: Growth cream-colored to brownish. Aerial mycelium white. Soluble pigment brown. Liquefaction slow.

Milk: Surface growth dark brown. Aerial mycelium white. Coagulation and slow peptonization.

Nitrate reduction: Positive.

Sucrose: No inversion.

Cellulose: No growth.

Habitat: Pineapple soil in Hawaii.

Remarks: This organism had been described by Waksman (1919) as *Actinomyces* 145, but never named before.

Type culture: IMRU 3067.

164. *Streptomyces nitrificans* Schatz *et al.*, 1954 (Schatz, A., Isenberg, H. D., Angrist, A. A., and Schatz, V. J. Bacteriol. **68:** 1–4, 1954).

Morphology: Sporulating hyphae straight, branched.

Most solid and liquid media: Growth gray, with a pink to buff reverse. No soluble pigment.

Blood agar: Growth brick-red. No hemolysis.

Potato: Growth wrinkled.

Nitrate reduction: Positive.

Milk: No coagulation; slow peptonization.

Starch: Hydrolysis.

Gelatin: No liquefaction.

Cellulose: Not attacked.

Remarks: *S. nitrificans* grows well on a variety of substrates, such as ethyl carbamate. With ammonia providing nitrogen in the basal medium, glucose, sucrose, mannitol, sorbitol, glycerol, ethanol, *n*-propanol, acetate, lactate, succinate, fumarate, and citrate permitted good growth. In a glucose containing medium, ammonia, nitrite, nitrate, urea, and guanidine were satisfactory sources of nitrogen. Several amino acids, purines, and miscellaneous other nitrogenous compounds, supplied alone or with glucose in the basal medium, supported growth.

The organism grew as well on carbamate when first isolated from a carbamate-enrichment culture as it did after serial transfer over a 2-year period on various simple and complex media containing no carbamate.

In addition to its apparently unique ability to grow on carbamate as sole substrate, this culture also produced nitrite from carbamate. It did not oxidize the carbamate nitrogen beyond the nitrite stage. Hirsch (1960) considers this organism as a *Nocardia* (*N. nitrificans*) capable of utilizing petroleum.

165. *Streptomyces nitrosporeus* Okami, 1952 (Okami, Y. J. Antibiotics (Japan) **5:** 477–480, 1952).

Morphology: Aerial mycelium straight, formed in clusters or tufts. Spores elliptical to oval.

Sucrose nitrate agar: Substrate growth colorless, grayish. Aerial mycelium blackish-gray.

Gelatin: Limited growth in liquefied zone. Liquefaction rapid. Soluble pigment yellowish-brown.

Milk: Growth cream-colored to brownish. Strong coagulation and peptonization.

Starch: Strong hydrolysis.

Cellulose: Attacked.

Nitrate reduction: Vigorous.

Tyrosinase reaction: Negative.

Production of H_2S: None.

Loeffler's serum media: Growth thin. Aerial mycelium gray. Soluble pigment limited. Rapid liquefaction of serum.

Carbon utilization: Utilizes arabinose, galactose, glucose, maltose, rhamnose, xylose, and glycerol; does not utilize sucrose, fructose, inulin, lactose, mannitol, raffinose, or sorbitol.

Antagonistic properties: Produces an antibiotic, nitrosporin (proactinomycin?).

Habitat: Soil in Japan.

Remarks: Resembles *S. griseolus* and *S. cellulosae*.

Type culture: IMRU 3728; ATCC 12,769.

166. *Streptomyces niveoruber* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 350, 1958).

Morphology: Long, straight sporophores, monopodially branched, forming open, regular spirals. Spores smooth (Pl. II k).

Glycerol nitrate agar: Growth light yellow or carmine-red. Aerial mycelium sparse, chalk-white.

Glucose-peptone agar: Growth white-yel-

low to carmine-red. Aerial mycelium abundant, white. Soluble pigment somewhat carmine-red.

Calcium malate agar: Growth light yellow to light carmine. No aerial mycelium.

Starch agar: Growth light yellow-red to carmine-red. Aerial mycelium abundant, white. Limited hydrolysis.

Potato: Growth light brown. Aerial mycelium powdery, chalk-white. Melanin-negative.

Gelatin: Growth carmine-red. Aerial mycelium sparse. Trace of liquefaction. No soluble pigment.

Milk: Pellicle light yellow. Aerial mycelium sparse. Coagulation limited; no peptonization.

Antagonistic properties: Produces antibiotic cinerubin.

Habitat: Soils in England and Germany.

167. *Streptomyces niveus* Smith *et al.*, 1956 (Smith, C. G., Dietz, A., Sokolski, W. T., and Savage, G. M. Antibiotics & Chemotherapy **6:** 135–142, 1956).

Morphology: Sporophores straight at the base, corkscrew-coiled at tip, occur in clusters and bear oblong spores.

Sucrose nitrate agar: Growth cream-colored. Aerial mycelium white. Soluble pigment yellow.

Calcium malate agar: Growth cream-colored. Aerial mycelium white. Soluble pigment yellow.

Nutrient agar: Growth cream-colored. Aerial mycelium trace, gray-white. Soluble pigment yellow.

Gelatin: Growth good. Liquefaction partial. No soluble pigment.

Nutrient starch agar: Growth yellow. Aerial mycelium cream-pink. Hydrolysis good.

Tyrosine agar: Soluble yellow pigment.

Milk: Ring on surface; flocculent growth at bottom. Positive peptonization.

Production of H_2S: Negative.

Carbon utilization: D-xylose, D-arabinose, rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, sucrose, lactose, cellobiose, raffinose, dextrin, inulin, soluble starch, glycerol, dulcitol, D-mannitol, D-sorbitol, inositol, salicin, sodium formate, sodium oxalate, sodium tartrate, sodium acetate, sodium citrate, and sodium succinate utilized. Phenol, cresol, and sodium salicylate not utilized.

Nitrate: No reduction.

Antagonistic properties: Produces streptonivicin, a form of novobiocin.

Habitat: Soil.

Remarks: According to Kuroya *et al.* (1958), this organism is related if not identical to *S. griseoflavus*.

168. *Streptomyces noboritoensis* Isono *et al.*, 1957 (Isono, K., Yamashita, S., Tomiyama, Y., Suzuki, S., and Sakai, H. J. Antibiotics (Japan) **10A:** 21–30, 1957).

Morphology: Aerial mycelium long and wavy; no regular spirals.

Sucrose nitrate agar: Growth colorless. Aerial mycelium slight. Soluble pigment absent or pale yellow.

Glucose-asparagine agar: Growth pale brown to dark brown. Aerial mycelium pale violet-gray. No soluble pigment or slightly brownish.

Nutrient agar: Growth flat, pale gray, smooth and restricted. No aerial mycelium. Soluble pigment dark red-brown.

Starch agar: Growth dry, wrinkled, pale grayish-brown. Aerial mycelium pale-gray, cottony. Weak diastatic action.

Gelatin: Growth dark brown. Soluble pigment dark brown. Liquefaction absent or slight.

Potato: Growth flat, wrinkled, black. Aerial mycelium grayish-white in some strains. Color of plug black.

Carbon utilization: Glucose, lactose, mannitol, trehalose, and raffinose well utilized. Utilization of arabinose, inositol, salicin, and xylose limited. Rhamnose and sucrose not utilized.

Antagonistic properties: Produces antibiotic homomycin-hygromycin.

Remarks: *S. noboritoensis* belongs to the group of chromogenic actinomycetes closely related to *S. cinnamonensis*, *S. flavochromogenes*, *S. phaeochromogenes*, *S. aureus*, and *S. tanashiensis*. They differ in spiral formation, pigmentation on synthetic media, nitrate reduction, and production of antibiotics.

169. *Streptomyces nodosus* Trejo, W. nov. sp.*

Morphology: Aerial mycelium forms open and closed spirals, the latter predominating as tightly knotted coils. Spores spherical to oval, 0.5 to 1.0 by 1.0 μ.

Sucrose nitrate agar: Growth white-greenish. Aerial mycelium pearl-gray to dawn-gray.

Nutrient agar: Substrate growth scant. No aerial mycelium. No soluble pigment. Melanin-negative.

Oatmeal agar: Growth black with a buff margin. Aerial mycelium deep olive-gray. Reverse: olivaceus to black with a peripheral ring of cream-buff to chamois. No soluble pigment.

Potato: Growth buff. Aerial mycelium light olive-gray. No darkening of plug.

Milk: Rapidly peptonized.

Gelatin: Rapidly hydrolyzed.

Nitrate reduction: Positive.

Starch: Strong hydrolysis.

Tyrosine: Utilized with no melanin formation.

Carbon utilization: Utilizes mannitol, inositol, rhamnose, xylose, D-fructose, trehalose, and melibiose. Does not utilize adonitol, sorbitol, arabinose, cellulose, sucrose, lactose, sodium acetate, esculin, or dextrin.

Antagonistic properties: Produces an antifungal antibiotic, amphotericin.

* Personal communication from Squibb Institute for Medical Research (1958).

Source: Isolated from soil in South America.

Remarks: This culture was specially described for this treatise. It appears to be closely related to *S. rutgersensis*.

170. *Streptomyces noursei* Hazen and Brown, 1950 (Hazen, E. L. and Brown, R. Science **112:** 423, 1950; Proc. Soc. Exptl. Biol. Med **76:** 93, 1951; Science **117:** 609, 1953).

Morphology: Sporophores produced as side branches of sterile aerial hyphae; occasionally produce open spirals and, according to Ettlinger *et al.* (1958), also some verticils. Spores round to oval, with thin long spines (Pl. I e).

Sucrose nitrate agar: Growth scanty, colorless, flat. No aerial mycelium.

Glucose-asparagine agar: Growth wrinkled, tan-colored, with gray and white knob-like projections. Reverse of growth dark gray. Aerial mycelium white, then reddish-gray, finally ash-gray; limited shell-pink diffusible pigment.

Glucose-peptone agar: Growth good, folded, brown. Aerial mycelium white, turning gray. Soluble pigment brown or pomegranate-purple.

Starch agar: Growth in form of discrete colonies. Aerial mycelium white in center, periphery colorless and embedded. Hydrolysis.

Potato: Growth folded. Aerial mycelium chalky white. At 35–36°C a reddish-purple pigment is formed.

Gelatin: Rapid liquefaction. Melanin-negative.

Milk: Coagulation, followed by peptonization.

Cellulose: Growth poor.

Nitrate: Traces of nitrite produced.

Production of H_2S: Negative.

Blood agar: Growth consists of convex, lobate colonies, with central perforation. Aerial mycelium heavy, chalky white. No hemolysis, but darkening of blood.

Antagonistic properties: Produces an antifungal agent, nystatin.

Type culture: IMRU 3771.

171. *Streptomyces novaecaesareae* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 111, 1916, **8:** 158, 1919).

Morphology: Aerial mycelium forms both straight and spiral (dextrorse) sporophores. Spores oval to elongate.

Sucrose nitrate agar: Growth gray, becoming bluish, glossy, much wrinkled. Aerial mycelium white, appears late. Soluble purple pigment formed.

Glucose-asparagine agar: Growth restricted, gray, becoming red.

Nutrient agar: Growth thin, cream-colored.

Potato: Growth wrinkled, cream-colored, turning yellowish. Melanin-negative.

Gelatin: Surface colonies small, cream-colored. Liquefaction slow.

Milk: Gray ring. Coagulation slow; peptonization slow.

Starch agar: Colonies restricted, circular, bluish-violet. Positive hydrolysis.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum, 37°C.

Antagonistic properties: Negative.

Remarks: At first this organism was designated as *A. violaceus-caesari*. This species is considered by Krassilnikov as synonymous with *A. violaceus* (Rossi-Doria) Gasperini. It appears to be related to *S. violaceoruber*.

172. *Streptomyces odorifer* (Rullman *emend.* Lachner-Sandoval, 1898) Waksman (Lachner-Sandoval, V. Ueber Strahlenpilze. Strassburg, 1898).

Morphology: Sporophores long, straight, branching, forming spirals. Spores spherical.

Sucrose nitrate agar: Growth cream-colored, with trace of brown. Aerial mycelium abundant, cream-colored.

Glucose-asparagine agar: Growth cream-colored to brownish. Aerial mycelium abundant, cream-colored. Soluble pigment faint brownish.

Nutrient agar: Growth folded, brown. Aerial mycelium white around edge. Soluble pigment faint brown.

Starch agar: Growth cream-colored to brown. Aerial mycelium abundant, cream-colored to straw-colored. No soluble pigment. Hydrolysis good.

Maltose-peptone agar: Foulerton and Price-Jones (1902) described growth as "raised, drab-colored, semi-translucent, the surface becoming reticulated; soluble pigment deep brown; gelatin liquefied, with light brown pigmentation."

Potato: Growth folded, brownish. Aerial mycelium cream-colored. Soluble pigment faint brown.

Gelatin: Surface ring cream-colored. Aerial mycelium thin, white. No soluble pigment. Liquefaction slow.

Milk: Surface ring colorless to brownish. No aerial mycelium. No coagulation; some peptonization.

Cellulose: Good growth.

Sucrose: Inversion.

Paraffin and fats: Good growth.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Odor: Strong, characteristic of soil.

Antagonistic properties: Some strains give positive effects, others are negative.

Habitat: Soil.

Type culture: IMRU 3334.

173. *Streptomyces oidiosporus* (Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 23, 1941).

Morphology: Sporophores straight or wavy, never forming spirals; short or long, frequently forming broom-shaped structures. Spores 1.0 to 1.8 by 0.5 to 1.0 μ, frequently appearing as double cocci or segmented spores (oidiospores).

Agar media: Growth red or rose to pale;

pigment insoluble in medium. Aerial mycelium poorly developed, velvety, rose-white.

Gelatin: Aerial mycelium weakly developed, frequently lacking; aerial hyphae short, rose-white. Liquefaction weak.

Milk: No coagulation; peptonization positive.

Starch: Rapid hydrolysis of starch.

Cellulose: No growth.

Nitrate reduction: Positive.

Antagonistic properties: None. Jolly (1956) obtained positive effects for his strain.

Habitat: Rarely found in soil.

Remarks: The organism resembles *S. ruber* and *S. longispororuber*. Some strains were obtained as variants of *Nocardia rubra*. Jolly (1956) reported the isolation of a strain of *S. oidiosporus* from an Italian soil.

174. *Streptomyces olivaceus* (Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. Soil Sci. **8:** 168, 1919).

This organism was first described as strain No. 206 by Waksman (1919). It was used by Jensen (1930) for comparison with his own isolates. It was studied more recently by Shinobu (1958) and Ettlinger *et al.* (1958).

Morphology: Sporophores branched monopodially, straight or somewhat wavy; no true spirals on most media; a few long, open spirals on calcium malate agar. Spores spherical and oval, 0.8 to 1.2 μ; surface smooth (Pl. III).

Sucrose nitrate agar: Growth abundant, yellow to olive-ocher, reverse yellow to almost black. Aerial mycelium ash-gray to light drab.

Glucose-asparagine agar: Growth yellow to light olive to olive-gray. Aerial mycelium light olive-gray to light brownish-gray with greenish tinge. No soluble pigment.

Calcium malate agar: Growth greenish-yellow to yellow. Aerial mycelium yellowish-white to yellowish-gray. Soluble pigment yellow.

Nutrient agar: Growth white, glistening. No soluble pigment.

Starch agar: Growth brownish-yellow to yellowish-green. Aerial mycelium brownish-white. Hydrolysis strong.

Potato: Growth abundant, much wrinkled, elevated, gray, turning sulfur-yellow on edge. Melanin-negative.

Gelatin: Liquefaction rapid. No soluble pigment.

Milk: Growth faint, pinkish; coagulation and peptonization rapid.

Cellulose: Growth good.

Mannase: Reaction strong, according to Shinobu (1958).

Nitrate reduction: Positive.

Production of H_2S: Negative.

Tyrosinase reaction: Although this organism has been considered as melanin-negative, Shinobu (1958) reported a positive reaction.

Temperature: Optimum 25°C.

Carbon sources: According to Shinobu (1958), *S. olivaceus* rapidly utilizes xylose, rhamnose, fructose, galactose, sucrose, lactose, and mannitol; slow utilization: trehalose, raffinose, and inositol.

Antagonistic properties: Various strains produce a variety of antibiotics, including streptomycin, olivacein, and granaticin.

Habitat: Very common in soil.

Remarks: Krassilnikov (1949) placed the organism in the *A. flavus* group. Ettlinger *et al.* (1958) considered the following organisms as belonging to *S. olivaceus: S. felleus, S. flavus, S. griseolus, S. halstedii, S. narbonensis, S. scabies* (sic), and *S. verne*.

Type culture: IMRU 3335.

175. *Streptomyces olivochromogenes* (Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. *Actinomyces* No. 205, Soil Sci. **8:** 106, 1919).

Morphology: Sporophores form numerous closed spirals. Spores oval or elliptical.

Sucrose nitrate agar: Growth white, spreading. Aerial mycelium ash-gray with brownish tinge. No soluble pigment.

Glycerol malate agar: Growth colorless. Aerial mycelium light grayish-olive to dark gray.

Glucose-asparagine agar: Growth abundant, natal-brown to almost black. Aerial mycelium white with gray tinge. Soluble pigment brownish.

Nutrient agar: Growth wrinkled, brown, becoming gray-green. Aerial mycelium white. Soluble pigment brown.

Starch agar: Growth transparent, spreading. Aerial mycelium buff-gray. Rapid hydrolysis.

Potato: Colonies small, wrinkled, black. No aerial mycelium. Soluble pigment black.

Gelatin: Surface growth cream-colored, spreading. Aerial mycelium white. Soluble pigment dark brown to deep olive-green. Slow liquefaction.

Milk: Dark brown ring. Coagulation and peptonization.

Cellulose: Growth faint.

Nitrate reduction: Faint reduction to nitrite.

Sucrose: Invertase positive with good growth.

Temperature: Optimum, 37°C.

Antagonistic properties: Positive.

Habitat: Soil, water, river mud.

Remarks: Ettlinger *et al.* (1958) considered this organism as a strain of *S. griseus*. Krassilnikov (1949) considered it as a variety of *A. chromogenes*.

176. *Streptomyces olivoreticuli* Arai *et al.*, 1957 (Arai, T., Nakada, T., and Suzuki, M. Antibiotics & Chemotherapy **7:** 435–442, 1957).

Morphology: Sporophores form primary and secondary verticils; secondary may also be formed as tip clusters. Spores spherical to oval.

Sucrose nitrate agar: Growth thin, yellow to brown. Aerial mycelium scant, later becoming cottony, white with yellowish tinge. Soluble pigment faint brown or absent.

Glucose-asparagine agar: Growth thin, light brown to olive-drab. Aerial mycelium cottony, white with faint yellow to grayish-pink tinge.

Nutrient agar: Growth limited, brownish. Aerial mycelium grayish-white. Soluble pigment light brown.

Gelatin: Surface growth poor. Liquefaction slow, later becoming rapid. Soluble pigment brown.

Blood agar: Strong hemolysis.

Potato: Growth wrinkled, dark brown. Aerial mycelium abundant, powdery, cream-colored to tea-green. Soluble pigment brown.

Milk: Ring on surface brown. Coagulation with limited peptonization. Soluble pigment brown.

Nitrate reduction: Negative.

Starch: Hydrolysis.

Cellulose: No decomposition.

Antagonistic properties: Produces antibiotic viomycin.

177. *Streptomyces olivoverticillatus* Shinobu, 1956 (Shinobu, R. Mem. Osaka Univ. B (N. S.) **5:** 84–93, 1956).

Morphology: Sucrose-ammonium agar most suitable for microscopic study. Primary and secondary verticils produced, branches issuing sometimes closely, near the top of the sporulating hyphae, forming cluster-like or tuft-like branches. Spores spherical to elliptical, 0.6 to 0.8 μ.

Sucrose nitrate agar: Trace of growth.

Glucose-asparagine agar: Growth thin, moderate, pale olive to pale dark yellow. Aerial mycelium thin, partially yellowish-gray.

Nutrient agar: Growth heavy, deep brown. Aerial mycelium olive-gray to yellow to green. Soluble pigment brown.

Potato: Growth heavy, brown. Aerial mycelium yellow-white to yellow-gray. Soluble pigment brown.

Milk: Growth brown. Aerial mycelium scant, yellow-white. Soluble pigment brown.

Gelatin: Liquefaction weak.

Starch: Rapid hydrolysis.

Tyrosinase reaction: None.

Nitrate reduction: Negative.

Cellulose: No growth.

Carbon utilization: Fructose and inositol utilized. Xylose, rhamnose, sucrose, lactose, raffinose, and mannitol not utilized.

Habitat: Soil in Japan.

178. *Streptomyces omiyaensis* Umezawa *et al.*, 1949 (Umezawa, H., Tazaki, T., Okami, Y., and Fukuyama, S. J. Antibiotics (Japan) **3:** 294–296, 1949).

Morphology: Aerial mycelium shows scant branching. Sporophores straight, no spirals. Spores 1.0 to 1.2 by 2 to 3 μ.

Sucrose nitrate agar: Growth thin, transparent, cream-colored to dark. Aerial mycelium absent, or scant, white. No soluble pigment.

Nutrient agar: Growth wrinkled, white to cream-colored. No aerial mycelium. No soluble pigment. Melanin-negative.

Starch agar: Colorless thin colonies, almost all submerged. No aerial mycelium. No soluble pigment. Hydrolysis.

Gelatin: Growth on surface white. No soluble pigment. Liquefaction slight in crateriform.

Potato: Growth white to cream-colored. No aerial mycelium. No soluble pigment.

Milk: Growth white. Peptonization rapid. Acid formed.

Antagonistic properties: Produces the antibiotic chloramphenicol.

Habitat: Soil.

Remarks: Related to *S. cacaoi*.

179. *Streptomyces orientalis* Pittenger and Brigham, 1956 (Pittenger, R. C. and Brigham, R. B. Antibiotics & Chemotherapy **6:** 642–647, 1956).

Morphology: Substrate growth made up of typical prostrate, much-branched mycelium. Aerial mycelium abundant if starch is used as carbon source. Straight or irregularly branched sporophores made up of cylindrical to ovoid spores, 0.7 to 1.0 by 1.4 to 1.8 μ.

Sucrose nitrate agar: Growth scant to moderate, pale cream color. Aerial mycelium trace of off-white. No pigment or pale yellowish-brown to light brown soluble pigment may be formed.

Glucose-asparagine agar: Growth moderate to good, cream-colored. Aerial mycelium pale to cream-colored, powdery. Soluble pigment pale greenish-yellow.

Glycerol malate agar: Growth pale cream to intense cream-yellow. Aerial mycelium whitish in color. No soluble pigment. Insoluble malate cleared in agar around growth.

Nutrient agar: Growth cream-colored. Aerial mycelium whitish. No soluble pigment.

Starch agar: Growth moderate, cream-colored to buff to brown. Aerial mycelium white, becoming pale cream and finally grayish. Soluble pigment cream-yellow, becoming pale brown. Hydrolysis limited.

Potato plug: Growth shows slightly rough surface. Aerial mycelium white. Slight to moderate amount of brown discoloration of plug.

Gelatin: Growth flocculent, not forming intact pellicle. Aerial mycelium scant, white. No soluble pigment. Liquefaction moderate.

Milk: Heavy wrinkled pellicle, with dull gray aerial mycelium. No coagulation. Peptonization begins in 11 to 14 days and is complete in 14 to 21 days. Very dark soluble pigment obscures litmus color.

Cellulose: Growth good.

Antagonistic properties: Antibiotic vancomycin produced.

Remarks: *S. orientalis* is most closely related to species intermediate between *S. albus* and *S. flavus*, such as *S. alboflavus*, *S. flavisporus*, and *S. longisporoflavus*. *S. albogavus* cannot utilize cellulose, hydrolyzes gelatin far less effectively than *S. orientalis*, but attacks starch readily. Milk is feebly

digested by *S. alboflavus* but rapidly hydrolyzed by *S. orientalis*. Production of aerial mycelium by the two cultures differs on several media.

180. *Streptomyces paraguayensis* (Almeida, 1940) nov. comb. (Almeida, F. Mycopathologia **2:** 201–203, 1940).

Morphology: Thin, ramified mycelial filaments; aerial mycelium consists of thicker and darker filaments, 1 μ in diameter. Gram-positive and nonacid-nonalcohol resistant.

Glucose-peptone agar: Growth hard, adhering to the medium; white with dark center, gradually changing to dark yellow to almost chocolate.

Nutrient agar: Growth rough, adhering to the medium; dark gray in color.

Potato: Colonies cerebriform, white; growth dry and friable.

Gelatin: Growth on surface. No liquefaction.

Milk: Surface membrane, the milk colored pink; no peptonization.

Habitat: Thoracic mycetoma; dark heavy grains.

181. *Streptomyces parvullus* Waksman and Gregory, 1954 (Waksman, S. A. and Gregory, F. J. Antibiotics & Chemotherapy **4:** 1050–1056, 1954).

Morphology: Sporophores long, monopodially branched, twisting into long closed spirals. Spores spherical, smooth (Pl. I g).

Sucrose nitrate agar: Growth abundant with yellow reverse. Aerial mycelium ash-gray. Soluble pigment yellow.

Glucose-asparagine agar: Growth yellow. Aerial mycelium abundant, gray. Soluble pigment yellow.

Nutrient agar: Growth yellowish, covered with thin white aerial mycelium. Soluble pigment yellow. Melanin-negative.

Potato: Growth orange-colored, covered with white to gray aerial mycelium. No soluble pigment.

Gelatin: Surface pellicle covered with heavy gray aerial mycelium. Liquefaction slow. Soluble pigment yellow.

Milk: Surface growth heavy, greenish-yellow. Aerial mycelium abundant, gray. Soluble pigment brown. No coagulation, very slow peptonization.

Production of H_2S: Negative.

Antagonistic properties: Produces actinomycin D.

Habitat: Soil.

Type culture: IMRU 3677.

182. *Streptomyces parvus* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 685–686, 1914).

Morphology: Sporophores straight, branched, or wavy; no true spirals; some strains, however, produce spirals. Spores oval, 0.9 to 1.3 by 1.2 to 1.8 μ.

Sucrose nitrate agar: Growth yellow, rose or red. Aerial mycelium light yellow to white-rose. Soluble pigment rose-colored to bright yellow.

Calcium malate agar: Small yellow colonies. Light yellow aerial mycelium.

Nutrient agar: Growth yellow. Aerial mycelium light yellow. Soluble pigment bright yellow.

Potato: Growth yellow to brown-yellow. Aerial mycelium white to yellow. Melanin-negative.

Gelatin: Growth yellow. Soluble pigment bright yellow. Liquefaction slow.

Milk: No coagulation; rapid peptonization.

Starch agar: Growth rose-colored. Aerial mycelium light gray. Hydrolysis positive.

Cellulose: Growth good, rose-colored. Aerial mycelium yellowish-gray.

Nitrate reduction: Weak.

Production of H_2S: Negative.

Antagonistic properties: Produces actinomycin.

Habitat: Soil.

Remarks: Ettlinger *et al.* (1958) con-

sidered *S. parvus* as belonging to the *S. griseus* series. According to Gause *et al.* (1957), *S. parvus* is a member of the series *Fradiae*.

Type culture: IMRU 3686.

183. *Streptomyces pelletieri* (Laveran, 1906) nov. comb. (Laveran, S. Compt. rend. soc. biol. **61:** 340, 1906).

Morphology: Growth red, smooth, consisting of small, dense, pink colonies. Mycelium nonsegmented, branched; hyphae slender, straight, and not very long. Aerial hyphae few, straight.

Glucose-asparagine agar: Growth in form of small, hard, red or purple adherent colonies. No soluble pigment.

Glucose agar: Growth poor, in form of minute, pink colonies.

Glycerol agar: Growth poor, as few moist, pink colonies.

Nutrient agar: Colonies minute, colorless, piled up into pale pink masses.

Potato: Growth sparse, yellowish-pink, irregularly piled up; later, abundant, small, rounded, pink masses. Aerial mycelium scant, white.

Blood agar: Colonies at first a few pinhead, cream-colored; no hemolysis. Later, colonies are dense, button-shaped, with narrow, fringed margin.

Dorset's egg medium: Growth abundant, wrinkled, pink skin with small discrete colonies at margin; later, surface rough, mealy, with considerable liquefaction.

Gelatin: Few pink flakes. At first slow, later almost complete liquefaction.

Milk: Soft curd; gradual peptonization.

Starch: No hydrolysis.

Production of H_2S: Negative.

Source: Mycetoma in Nigeria.

Remarks: In the original description of this culture by Laveran, the organism was called *Micrococcus pelletieri*, because no mycelium was seen, only coccoid bodies. *N. indica* was regarded as identical by Pinoy. *N. genesii* Froes was described as closely allied (Erikson, 1935); the distinction was founded upon the fact that the red grains were smaller and much more numerous. *A. africanus* is considered as a synonym of this organism. According to Mariat (1958), *S. pelletieri* hydrolyzes gelatin, serum albumin, casein, and egg albumin; it utilizes urea but not $(NH_4)_2SO_4$ and KNO_3 as nitrogen sources; it does not utilize xylose, galactose, maltose, starch, mannitol, or paraffin as carbon sources. The species *S. africanus* is indistinguishable from *S. pelletieri*.

184. *Streptomyces pentaticus* Umezawa and Tanaka, 1958 (Umezawa, S. and Tanaka, Y. J. Antibiotics (Japan) **11A:** 26–29, 1958).

Morphology: Straight sporophores produce primary and secondary verticils. Spores can scarcely be observed.

Sucrose nitrate agar: Growth poor, transparent, penetrates deeply into medium. No aerial mycelium. No soluble pigment.

Glucose-asparagine agar: Growth colorless, becoming purplish-pink to dull red-purple, deep into medium. Aerial mycelium white, sometimes pink. Soluble pigment faint brown.

Calcium malate agar: Growth red, irregular margin. No aerial mycelium. Soluble pigment faint brown.

Nutrient agar: Growth wet, colorless or brownish-white. No aerial mycelium. Soluble pigment brown.

Starch agar: Growth colorless or pale yellow, penetrates deeply into medium. Aerial mycelium white, partially pinkish cottony colonies. No soluble pigment. Starch hydrolyzed.

Gelatin: Growth consists of reddish colonies produced on surface. No aerial mycelium. Soluble pigment deep brown. Rapid liquefaction.

Potato: Growth wrinkled, wet, grayish-brown. No aerial mycelium. Soluble pigment brownish-black.

Milk: Surface ring dull yellow. Coagulation and peptonization.

Antagonistic properties: Produces an antifungal polyenic antibiotic, pentamycin.

Habitat: Soil in Japan.

Remarks: Resembles *S. rubrireticuli*, which differs from the strain producing pentamycin in the following ways: spirals are formed; growth on nutrient agar is red; growth on milk is abundant and red; cellulose and sucrose are utilized.

185. *Streptomyces phaeochromogenes* (Conn, 1917) Waksman and Henrici, 1948 (Conn. H. J. N. Y. Agr. Expt. Sta. Tech. Bull. 60, 1917).

This culture has been studied by Conn (1917), Waksman (1919), Jensen (1931), Krassilnikov (1949), Kutzner (1956), and Ettlinger *et al.* (1958).

Morphology: Sporophores form narrow, open, elongated, sinistrorse spirals (Conn, Waksman, Jensen, Krassilnikov). Kutzner (1956) examined 25 strains belonging to this species; only five of them produced spirals. Ettlinger *et al.* (1958) could not find any spirals on any of the strains obtained from various culture collections. Spores spherical to short rods; surface smooth (Pl. I d).

Sucrose nitrate agar: Growth brown to almost black. Aerial mycelium abundant, white with brownish shade. Soluble pigment brown to dark brown.

Calcium malate agar: Growth buff to brown. Aerial mycelium white. Soluble pigment brown.

Nutrient agar: Growth gray to brown, later turning nearly black. Aerial mycelium white to gray, often absent. Soluble pigment deep red-brown.

Starch agar: Growth brown. Hydrolysis medium.

Potato: Growth brown to almost black. No aerial mycelium. Soluble pigment dark brown to black.

Gelatin: Surface growth abundant, spreading, cream-colored, becoming brown. Liquefaction slow. Soluble pigment brown.

Milk: Dark, almost black ring: coagulation with slow peptonization.

Nitrate: Reduction limited.

Production of H_2S: Positive.

Temperature: Optimum 25°C.

Antagonistic properties: Strong.

Habitat: Soil.

Type culture: IMRU 3338.

186. *Streptomyces phaeopurpureus* Shinobu, 1957 (Shinobu, R. Mem. Osaka Univ., B. Nat. Sci. **6:** 63–67, 1957).

Morphology: Substrate mycelium monopodial, 0.4 to 0.6 μ in diameter; no fragmentation. Aerial mycelium straight, usually short. Spores spherical to elliptical, 0.6 to 0.8 μ; rarely 1 μ.

Sucrose nitrate agar: Growth good, brown to dark red. No aerial mycelium. Soluble pigment brown.

Glycerol malate agar: Growth good, orange to purple. Aerial mycelium powdery, yellowish-gray to pinkish-gray. Soluble pigment red-purple to brown-purple.

Glucose-asparagine agar: Growth moderate, orange to red-brown. Aerial mycelium moderate, in patches, pinkish-gray. Soluble pigment reddish-orange to reddish-brown.

Nutrient agar: Growth good, deep brown. No aerial mycelium. Soluble pigment brown to deep reddish-brown.

Potato plug: Growth wrinkled, reddish to yellowish-brown. Aerial mycelium absent or scant, light brownish-gray. Plug colored brown.

Milk: Growth in form of deep brown ring. Soluble pigment brown. No coagulation; peptonization uncertain.

Tyrosinase reaction: Positive.

Gelatin: Liquefaction fairly strong.

Diastase: Weak.

Cellulose: Negative.

Nitrate reduction: Negative.

Carbon sources: Utilizes xylose, rhamnose,

fructose, sucrose, lactose, raffinose, mannitol, and inositol.

Habitat: Soil in Japan.

187. *Streptomyces phaeoviridis* Shinobu, 1957 (Shinobu, R. Mem. Osaka Univ., B. Nat. Sci. **6:** 67–70, 1957).

Morphology: Growth monopodial, hyphae 0.4 to 0.6 μ in diameter, no fragmentation. Aerial mycelium short; monopodial branching; some spirals, sinistrorse, 1 to 3 turns. Spores elliptical, 0.6 to 0.8 μ.

Sucrose nitrate agar: Growth pale yellow to dark brown. Aerial mycelium scant, brownish-white. Soluble pigment yellow to brown to dark blue.

Malate-glycerol agar: Growth yellowish-brown to dark brown. Aerial mycelium scant, white to brownish-white. Soluble pigment brown to dark brown with blue tinge.

Glucose-asparagine agar: Growth thin brown to yellow-orange; aerial mycelium scant, white to brownish-white. Soluble pigment pale brown to yellow-orange.

Nutrient agar: Growth thin, yellow-orange to brown. No aerial mycelium. Soluble pigment brown to dark red with purple tinge.

Potato: Growth poor, pale brown. No aerial mycelium. Soluble pigment uncertain, probably pale brown.

Milk: Growth good, pale yellow, partially blue, sometimes grayish-green. Soluble pigment absent or pale orange. Coagulation and peptonization.

Tyrosinase reaction: Negative.

Gelatin: Liquefaction variable.

Diastase: Strong.

Nitrate reduction: Negative.

Cellulose: No growth.

Carbon sources: Utilizes xylose, rhamnose, sucrose, fructose, raffinose, and mannitol; lactose and inositol uncertain.

Habitat: Soil in Japan.

188. *Streptomyces pilosus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 347, 1958).

Morphology: Sporophores monopodially branched, with long, regular, open spirals. Spores covered with fine long hair.

Glycerol nitrate agar: Growth yellow to yellow-brown. Aerial mycelium powdery, chalk-white to gray-blue.

Glucose-asparagine agar: Growth light yellow. Aerial mycelium powdery, white to ash-gray.

Calcium malate agar: Growth white-yellow to yellow-brown. Aerial mycelium white-yellow to white-gray.

Starch agar: Growth yellow-brown to red-yellow. Aerial mycelium scant, white to ash-gray. Limited hydrolysis.

Gelatin: Growth yellow-brown. Aerial mycelium powdery, chalk-white. Liquefaction slow. Soluble pigment dark brown.

Potato: Growth golden yellow. Aerial mycelium ash-gray. Soluble pigment dark brown.

Milk: Surface growth white-gray to grayish-blue. Aerial mycelium ash-gray. No coagulation; peptonization weak.

Antagonistic properties: Positive.

Habitat: Soil from Rome, Italy.

189. *Streptomyces platensis* Pittenger and Gottlieb, 1954 (Pittenger, R. C. and Gottlieb, D. Brit. Pat. 713,795, August 18, 1954*).

Morphology: Aerial mycelium forms loose to tight spirals on its sporophores. Spores ovoid, 0.7 to 0.9 by 0.8 to 1.2 μ.

Sucrose nitrate agar: Substrate growth deep olive, reverse becoming dark olive. Aerial mycelium pale smoke-gray with tufts of white; areas of black pigmented aerial growth may also be found, giving effect of a mosaic.

Glucose - asparagine agar: Substrate growth ochraceous-buff becoming tawny. Aerial mycelium white becoming grayish-olive to almost black. Soluble pigment absent or slight, brown.

* Supplemented by personal communication.

Calcium malate agar: Growth ochraceous-salmon, becoming cinnamon-buff. Aerial mycelium quaker-drab with areas of black and white. Soluble pigment slight, greenish-yellow.

Nutrient agar: Poor substrate growth, cream-yellow becoming buff to drab. No aerial mycelium. Slight soluble brown pigment.

Starch agar: Growth cream- to buff-colored. Aerial mycelium white, becoming mouse-gray with patches of black. Slow hydrolysis.

Potato: Excellent growth. Aerial mycelium white to pale mouse-gray. Soluble pigment brown.

Gelatin: Very slow liquefaction. Melanin-negative.

Milk: Growth scant, forming partial ring at surface. No coagulation or peptonization.

Blood: Hemolysis.

Cellulose: Growth slight. Aerial mycelium gray to black.

Nitrate: Reduction to nitrite, especially with starch as source of carbon.

Carbon utilization: Starch, malic acid, inositol, sodium succinate, sodium citrate, sorbitol, mannitol, maltose, arabinose, lactose, galactose, fructose well utilized. Dulcitol, raffinose, cellulose, sodium formate, sodium tartrate, xylose poorly utilized. Asparagine, rhamnose, *o*-cresol, *m*-cresol, sodium acetate, inulin, sodium salicylate not utilized.

Antagonistic properties: Produces oxytetracycline.

Remarks: Tresner and Backus (1956) considered this organism as a variant of *S. hygroscopicus* rather than a separate species. Ettlinger *et al.* (1958) came to similar conclusions.

190. *Streptomyces pluricolor* (Berestnew, 1897 *emend.* Krassilnikov, 1941) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 17, 1949).

Morphology: Sporophores produce numerous spirals, with 3 to 5 turns (sinistrorse). Spores oval, 0.9 by 0.7 μ.

Synthetic agar: Growth at first pigmented yellow-red, later becoming blue to blue-green. Aerial mycelium white-gray. The blue pigment dissolves into the medium.

Nutrient agar: Soluble pigment greenish, fluorescent.

Potato: Growth and soluble pigment sharp blue.

Gelatin: Liquefaction rapid.

Milk: Peptonization positive; no coagulation.

Starch: Hydrolysis.

Cellulose: No growth.

Nutrient broth: Soluble pigment green, fluorescent.

Sucrose: Inversion.

Antagonistic properties: None.

Habitat: Soil.

Remarks: Closely related to *S. violaceoruber*. *A. pluricolor diffundens* Berestnew is considered by Krassilnikov as a synonym.

191. *Streptomyces pluricolorescens* Okami, Y. and Umezawa, H.* n. sp.

Morphology: Aerial hyphae not flexuous, few branches; no spirals.

Glycerol nitrate agar: Growth at first colorless and yellowish, then yellowish-brown with reddish tone. Aerial mycelium white to olive or pinkish. Soluble pigment slightly yellowish-brown or light wine-color with aging.

Glucose–asparagine agar: Growth at first colorless and yellowish, then yellowish-brown with reddish tone. Aerial mycelium white to olive or pinkish. Soluble reddish-purple pigment occasionally produced.

Calcium malate agar: Same as on glycerol nitrate agar.

Nutrient agar: 37°C. Colorless or slight yellowish-brown growth with fine wrinkles. Aerial mycelium white. Soluble pigment absent at first, later a brown pigment appears.

* Personal communication from Okami.

Potato plug: Growth colorless, then slightly yellowish or brownish. Aerial mycelium white to olive-colored. Color of plug unchanged. Melanin-negative.

Gelatin: 18–20°C. Growth colorless. No aerial mycelium. Soluble pigment slightly yellowish-brown. Gelatin liquefied.

Milk: 37°C. Colorless to slight yellowish growth. No aerial mycelium. Coagulation with acid reaction, then peptonization.

Starch: Hydrolysis weak to medium.

Carbon utilization: Good growth with arabinose, dextrin, fructose, galactose, glucose, glycerol, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbose, starch, sucrose, xylose, and sodium succinate. Scant growth with esculin, inositol, lactose, sorbitol, sodium acetate, and citrate.

Antagonistic properties: Produces antitumor substances pluramycin A and B (Maeda *et al.*, 1956).

Remarks: This culture is said to be related to *S. vinaceus*, but it does not produce blue-red pigment in reverse of growth on nutrient agar.

192. *Streptomyces poolensis* (Taubenhaus, 1918) Waksman (Taubenhaus, J. J. J. Agr. Research **13:** 446, 1918).

Morphology: Sporophores straight. Spores oval to elliptical.

Sucrose nitrate agar: Growth thin, colorless. Aerial mycelium white to gray.

Glucose-asparagine agar: Growth abundant, glossy, light brown.

Nutrient agar: Growth translucent, yellowish to brown. Soluble pigment brown.

Potato: Growth thin, reddish-brown. Soluble pigment purplish.

Gelatin: Liquefaction, with small, brownish flakes in fluid.

Milk: Brownish ring. Coagulation and peptonization.

Starch: Growth restricted, cream-colored. No hydrolysis.

Nitrate reduction: Positive.

Antagonistic properties: Positive.

Habitat: Sweet-potato disease known as "pox."

193. *Streptomyces praecox* (Millard and Burr, 1926) Waksman and Henrici, 1948 (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores produce short, open spirals. Spores spherical or oval, 0.8 μ in diameter.

Sucrose nitrate agar: Growth thin, colorless. Aerial mycelium gray to olive-buff. On continued cultivation, aerial mycelium tends to become white.

Nutrient agar: Growth colorless. Aerial mycelium white.

Starch media: Growth thin, cream-colored. Aerial mycelium white with greenish tinge. Hydrolysis positive.

Potato: Growth lichenoid, cream-colored to light brown. Aerial mycelium white to olive-buff. Soluble pigment olive-buff to drab. On continued cultivation, no soluble pigment produced.

Gelatin: Growth good. Aerial mycelium white. Liquefaction medium. Melanin-negative.

Milk: Surface growth cream-colored, in form of ring. Aerial mycelium white. Coagulation slow; peptonization rapid.

Nitrate: Reduction variable.

Cellulose: Good growth, colorless. Aerial mycelium dark gray.

Tyrosinase reaction: Negative.

Production of H_2S: Negative.

Temperature: Grows well at 37.5°C.

Odor: Very strong.

Antagonistic properties: Represses growth of *S. scabies*.

Habitat: Knoblike scab of potatoes.

Remarks: According to Ettlinger *et al.* (1958), this organism belongs to the *S. griseus* series. Hoffmann (1958) described a culture of *S. praecox* that produced a light to dark gray aerial mycelium and many spirals; nonchromogenic.

Type culture: IMRU 3374.

194. *Streptomyces praefecundus* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight, frequently forming brushes. Spores spherical to oval, 0.8 by 0.85 μ.

Sucrose nitrate agar: Growth good, cream-colored. Aerial mycelium cottony, olive-buff. Soluble pigment cream-colored.

Nutrient potato agar: Growth lichenoid, gray. Aerial mycelium smooth, white to yellowish. Soluble pigment golden brown.

Potato: Growth good, wrinkled. Aerial mycelium white to yellowish to olive-buff. Soluble pigment gray to brown.

Gelatin: Surface growth good. Aerial mycelium white. Soluble pigment light pink to dark golden brown. Liquefaction rapid.

Milk: Surface growth good. Aerial mycelium scant, white. Coagulation and peptonization.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab and soil.

195. *Streptomyces prasinophilus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 345, 1958).

Morphology: Sporophores monopodially branched, long, straight, with open spirals, usually 1 to 3 coils. Spores covered with long, fine hair (Pl. II, K).

Glycerol nitrate agar: Growth red or red-brown. Aerial mycelium leek-green with white spots. Soluble pigment pink.

Glucose-asparagine agar: Growth brick-red. Aerial mycelium white to leek-green. Soluble pigment brick-red.

Glycerol malate agar: Growth brick-red. Aerial mycelium leek-green. Soluble pigment pink.

Glucose-peptone agar: Growth brick-red.

Starch-KNO_3 agar: Growth pink. Aerial mycelium white to leek-green. Soluble pigment light pink. Good hydrolysis.

Gelatin: Bottom flakes red to yellowish, later brick-red. Slow liquefaction. No soluble pigment. Melanin-negative.

Potato: Growth slow, flesh-red. No soluble pigment.

Milk: Strong coagulation and peptonization.

Antagonistic properties: Weak activity against gram-positive bacteria.

Habitat: Soil in Mallorca, Spain.

196. *Streptomyces prasinus* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 343, 1958).

Morphology: Sporophores monopodially branched, long, straight, with open spirals, forming 1 to 2 coils. Spores covered with short spines (Pl. II, K).

Glycerol nitrate agar: Growth colorless. Aerial mycelium grass-green, later dark green.

Glucose-asparagine agar: Growth whitish-yellow.

Glycerol malate agar: Growth copper-red. Aerial mycelium velvety, leek-green.

Gelatin: Growth limited, whitish-yellow. No soluble pigment. Liquefaction positive.

Starch agar: Growth reddish-brown. Aerial mycelium leek-green. Strong hydrolysis.

Potato: Growth limited, light brown. Aerial mycelium leek-green. Melanin-negative.

Milk: Heavy pellicle. Aerial mycelium whitish-gray to greenish-gray. No coagulation; slow peptonization.

Antagonistic properties: None.

Habitat: Soils in Mallorca and Belgian Congo.

197. *Streptomyces pseudogriseolus* Okami *et al.*, 1955 (Okami, Y., Utahara, R., Oyagi, H., Nakamura, S., and Umezawa, H. J. Antibiotics (Japan) **8A:** 126–131, 1955).

Morphology: Sporophores produce numerous closed spirals. Spores oval to cylindrical, 0.8 to 1.2 by 1.0 to 1.5 μ.

Glycerol nitrate agar: Growth colorless to

grayish-buff. Aerial mycelium grayish-buff, powdery. No soluble pigment.

Nutrient agar: Growth colorless. Aerial mycelium white, thin. Soluble pigment absent or slight, brown.

Potato: Growth colorless to slightly yellowish, elevated, wrinkled. Aerial mycelium white, cottony to velvety. No soluble pigment.

Milk: Surface growth orange. Aerial mycelium velvety, white. Coagulation and peptonization completed in 25 to 30 days.

Gelatin: Growth yellowish-brown. Aerial mycelium white to grayish. Soluble pigment brownish. Liquefaction weak to medium. Melanin-negative.

Starch: Hydrolysis strong.

Carbon utilization: Utilizes arabinose, dextrin, *iso*-dulcitol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, rhamnose, salicin, starch, sucrose, sodium acetate, sodium citrate, and sodium succinate. Does not utilize esculin, raffinose, sorbitol, or sorbose (inulin).

Antagonistic properties: Produces xanthomycin-like substance.

Habitat: Isolated from soil in Japan.

Remarks: This culture resembles *S. griseolus*, but it differs in spiral formation and growth on potato and milk media. These differences may not be enough to warrant establishing a new species, but the production of xanthomycin is a property not found in the culture liquid of *S. griseolus*.

Type culture: ATCC 12,770.

198. *Streptomyces purpureofuscus* Yamaguchi and Saburi, 1955 (Yamaguchi, T. and Saburi, Y. J. Gen. Appl. Microbiol. **1:** 201–235, 1955).

Morphology: Aerial hyphae long and straight; on synthetic and starch agars, they show a tuft-forming tendency at the margin. Spirals not produced. Spores cylindrical, 1.1 to 2.2 by 0.7 to 1.1 μ.

Sucrose nitrate agar: Growth thin, colorless, later becoming purple to dark purple; this property may be lost after repeated transfer, in which case growth remains white to light purple. Aerial mycelium powdery, white, later smoke-gray. Soluble pigment faint purple.

Glycerol malate agar: Growth brownish to purplish-brown. Aerial mycelium white, smoke-gray to light grayish-olive. Soluble pigment light brownish-purple.

Nutrient agar: Growth wrinkled, colorless. Aerial mycelium absent, or scant, white. Soluble pigment changes from reddish-brown to dark vinaceous-brown.

Starch agar: Growth yellowish-brown to dark olive-buff; sometimes with hygroscopic, black patches. Aerial mycelium velvety, at first white, later olive-gray. Usually no soluble pigment is produced, but sometimes faint pinkish-purple is seen. Strong hydrolysis.

Potato: Growth vigorous, finely wrinkled, at first purplish-brown or yellowish-brown, later becoming black. Aerial mycelium abundant, powdery, grayish. Soluble pigment purplish.

Gelatin: Growth dark brownish-gray. Aerial mycelium coarse, powdery, grayish-white. Soluble pigments yellowish-brown to brown and a more diffusible yellowish-green. Strong liquefaction.

Milk: Growth at first dull reddish-brown, later purplish-brown. Soluble pigment faint purplish and more diffusible faint yellowish. Coagulation and peptonization.

Cellulose: No growth.

Carbon utilization: D-xylose, L-arabinose, D-galactose, sucrose, lactose, raffinose, sodium succinate readily utilized. L-rhamnose, inulin, mannitol, sorbitol, inositol, acetate, and citrate not utilized.

Antagonistic properties: Active against gram-positive and acid-fast bacteria; possesses antitrichomonal activity.

Remarks: Related to *S. vinaceus*, *S. cylindrosporus*, and *S. purpureochromogenes*.

199. *Streptomyces purpurascens* Lindenbein, 1952 (Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Sporophores long, straight, with open and closed spirals, 2 to 5 turns as side branches. Spores covered with long spines (Pl. I b). A detailed electron microscope study of this organism has been made by Petras (1959).

Glycerol nitrate agar: Growth carmine-red to purple. Aerial mycelium cottony, white to purplish. Soluble pigment brown-red.

Glucose-asparagine agar: Growth carmine-red to purple. Aerial mycelium white to pinkish. Soluble pigment orange to carmine-red.

Glycerol malate agar: Growth carmine-red. Aerial mycelium white. Soluble pigment orange to brick-red.

Nutrient agar: Growth light brown, with dark brown reverse. Aerial mycelium white. Soluble pigment dark brown. Melanin-positive.

Glucose-peptone agar: Growth lichenoid, red to red-brown. Aerial mycelium white. Soluble pigment light brown.

Starch media: Growth light carmine to yellow-red. Aerial mycelium white. No soluble pigment. Hydrolysis strong.

Potato: Growth brownish to reddish. Aerial mycelium white to gray. No soluble pigment. (Kutzner (1956) observed on six strains a gray to black pigment on potato plug.)

Gelatin: Growth light brown. Aerial mycelium white. Soluble pigment red-brown. Liquefaction medium.

Milk: Growth red to dark brown. Aerial mycelium white. No proteolysis.

Cellulose: Growth white to red.

Production of H_2S: Positive.

Antagonistic properties: Produces rhodomycin.

Remarks: On continued growth on synthetic media, the culture may lose the property to produce the typical pigment. It can be regained, however, by growth on organic media. This organism is considered by Corbaz *et al.* (1957) as a synonym of *S. bobiliae*, except that the latter lost the property of producing aerial mycelium or spores. Lindenbein (1952) and Frommer (1959) obtained colorless mutants from *S. purpurascens*.

Type culture: IMRU 3660.

200. *Streptomyces purpureochromogenes* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 113, 1916; **8:** 132, 1919).

Morphology: Long sporophores produce few imperfect spirals. Spores spherical, 0.75 to 1.0 μ in diameter.

Sucrose nitrate agar: Growth restricted, smooth, gray, becoming brown with purplish tinge; center raised, margin yellow. Aerial mycelium dark brown to dark gray.

Glucose-asparagine agar: Growth abundant, gray, becoming brown to dark brown.

Nutrient agar: Growth gray to brownish, becoming dark brown, almost black. Soluble pigment dark brown. Melanin-positive.

Potato: Growth orange to orange-red.

Gelatin: Surface growth slow, brownish. Liquefaction slow.

Milk: Dark brown ring. Coagulation and slow peptonization.

Starch media: Colonies small, dark brown. Slight hydrolysis.

Cellulose: Moderate growth.

Sucrose: Inversion.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Temperature: Optimum 25°C.

Antagonistic properties: Active against various bacteria.

Habitat: Soil.

Type culture: IMRU 3343.

201. *Streptomyces putrificus* (Nikolaieva, 1915) Waksman (Nikolaieva, E. Arch. Biol. Nauk. **18:** 229, 1914).

Morphology: Sporophores spiral-shaped. Spores spherical.

Nutrient agar: Growth colorless to grayish. Aerial mycelium white. No soluble pigment.

Potato: Growth folded, sulfur-yellow. Aerial mycelium chalk-white. No soluble pigment. Melanin-negative.

Milk: Pellicle heavy. Aerial mycelium white. Peptonization gradual without previous coagulation.

Loeffler's serum: Growth yellow. No aerial mycelium. Serum liquefied and colored yellowish-brown.

Odor: Strong, putrefactive.

Habitat: Spring water.

Remarks: Decomposes proteins energetically, with the formation of bad-smelling products (H_2S, NH_3). Morphological properties given by Krassilnikov (1949), who considers this organism as a member of the *A. albus* group.

202. *Streptomyces pyridomyceticus* Okami *et al.*, 1957 (Okami, Y., Maeda, K., and Umezawa, H. J. Antibiotics (Japan) **7A:** 55–56, 1954; **10A:** 172, 1957).

Morphology: Sporophores form flexible, open spirals. Spores of irregular size.

Glycerol nitrate agar: Growth colorless to dark. Aerial mycelium thin, white, sometimes gray to brownish-gray. No soluble pigment.

Nutrient agar: Growth colorless. Aerial mycelium absent, or scant, white. No soluble pigment.

Potato: Growth wrinkled, dark yellowish-brown. Aerial mycelium absent, or later white. No soluble pigment.

Gelatin: Growth colorless. Aerial mycelium white, sometimes grayish. No soluble pigment. No liquefaction.

Starch: Hydrolysis.

Milk: Growth yellowish, in the form of surface ring. No aerial mycelium. Coagulation and peptonization absent or very slow.

Blood: No hemolysis.

Nitrate reduction: Negative.

Carbon utilization: Utilizes arabinose, dextrin, fructose, galactose, glucose, glycerol, maltose, xylose, and sucrose. Does not utilize dulcitol, esculin, inulin, lactose, mannose, raffinose, rhamnose, salicin, sorbitol, sodium citrate, sodium acetate, and sodium succinate.

Habitat: Soil in Japan.

Antagonistic properties: Produces antibiotic pyridomycin.

Remarks: Isolated by means of chlortetracycline-containing agar. Related to *S. cacaoi* and *S. flocculus*, as well as to *S. hygroscopicus*. Above description was first given under the name *S. albidofuscus*. It was later found that this name was preempted by Neukirch and Berestnew, and was, therefore, changed to *S. pyridomyceticus*.

203. *Streptomyces rameus* Okami *et al.*, 1959) (Okami, Y., Suzuki, M., Takita, T., Ohi, K., and Umezawa, H. J. Antibiotics (Japan) **12A:** 257–262, 1959).

Morphology: Aerial mycelium forms incomplete spirals or loops or hooks. Spores oval to oblong.

Glycerol nitrate agar: Growth yellow. Aerial mycelium white. Soluble pigment absent or yellowish.

Glucose-asparagine agar: Growth yellowish. Aerial mycelium white. Soluble pigment absent or yellowish.

Calcium malate agar: Growth colorless to yellowish. Aerial mycelium off-white. No soluble pigment.

Nutrient agar: Growth colorless to brownish. Aerial mycelium white. Soluble pigment absent or slight brownish.

Starch agar: Growth colorless to yellowish. Aerial mycelium white. Soluble pigment yellowish. Hydrolysis weak to medium.

Potato: Growth brownish. Aerial mycelium white. Soluble pigment brown to black.

Gelatin: Growth brownish. Aerial mycelium white. Soluble pigment brown. Liquefaction doubtful.

Milk: Coagulation weak; peptonization doubtful.

Nitrate reduction: Negative.

Carbon sources: Utilizes arabinose, dextrin, fructose, galactose, glucose, glycerol, inositol, maltose, mannitol, mannose, raffinose, starch, and sucrose. Lactose and xylose gave less response. Poor growth on inulin, rhamnose, salicin, sorbitol, sorbose, sodium acetate, and sodium citrate.

Antagonistic properties: Produces streptomycin.

Remarks: Related to *S. alboflavus*, *S. xanthophaeus*, and *S. orientalis*.

204. *Streptomyces ramnaii* Bhuiyan and Ahmad, 1956 (Bhuiyan, A. M. and Ahmad, K. Ann. Biochem. Exptl. Med. India **16:** 101–104, 1956).

Morphology: Open spirals, with 2 or 3 turns. Spores spherical, 0.8 μ in diameter.

Sucrose nitrate agar: Growth whitish. Aerial mycelium white to pale rose. No soluble pigment.

Glucose-asparagine agar: Growth colorless to pale rose. Aerial mycelium white, later pale rose.

Calcium malate agar: Growth smooth, cream-colored. Aerial mycelium white. Medium becomes clear.

Nutrient agar: Growth cream-colored, becoming light brown. Aerial mycelium powdery, white. Soluble pigment slight brown coloration. Melanin-negative.

Starch agar: Growth cream-colored to yellowish brown. No aerial mycelium. No soluble pigment. Hydrolysis rapid.

Potato: Growth abundant, cream-colored. Aerial mycelium white, turning pale rose.

Potato nutrient agar: Growth rapid, colorless to cream-colored. Aerial mycelium deep rose. Soluble pigment deep reddish-brown to almost red.

Gelatin: Growth cream-colored. No aerial mycelium. Soluble pigment absent or light brown. Liquefaction medium.

Milk: Growth cream-colored. No aerial mycelium. Coagulation, followed by peptonization. Reaction acid.

Nitrate reduction: Positive.

Cellulose: Growth good.

Optimum temperature: 37°C.

Antagonistic properties: Produces antibiotic ramnacin.

205. *Streptomyces ramulosus* Ettlinger *et al.*, 1958 (Ettlinger, L., Gäumann, E., Hütter, R., Keller-Schierlein, W., Kradolfer, F., Neipp, L., Prelog, V., and Zähner, H. Helv. Chim. Acta **41:** 216–219, 1958).

Morphology: Sporophores monopodially branched, straight with many side branches. Spores smooth (Pl. II h).

Glycerol nitrate agar: Growth at first carmine-red, later turning greenish-brown. Aerial mycelium ash-gray with greenish tinge. Substrate pigmented carmine-red.

Glucose-asparagine agar: Growth yellowish-red. Aerial mycelium gray. Substrate carmine-red.

Calcium malate agar: Growth light yellow. Aerial mycelium chalky white to gray with greenish tinge.

Glucose-peptone agar: Growth yellowish-red, partly greenish to greenish-black. Aerial mycelium powdery, ash-gray. Substrate greenish to brownish-black.

Starch agar: Growth light yellow. Substrate light carmine. Gradual hydrolysis.

Potato: Growth yellowish-red. Aerial mycelium chalk-white to ash-gray. Substrate carmine-red.

Gelatin: Growth light yellow. Soluble pigment light brown. No liquefaction.

Milk: Light yellow pellicle. No aerial mycelium. Coagulation limited; peptonization good.

Carbon utilization: Glucose, L-xylose, D-fructose, sucrose, inulin, D-sorbitol well utilized. Does not utilize raffinose, L-arabinose, D-mannitol, mesoinositol. Questionable utilization of L-rhamnose, salicin.

Antagonistic properties: Produces antibiotic acetomycin, active against gram-posi-

tive and gram-negative bacteria, as well as against trichomonads and amoebae.

206. *Streptomyces resistomycificus* Lindenbein, 1952 (Lindenbien, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: Sporophores long, with curling tips. Spores short, oval.

Glycerol nitrate agar: Growth yellow-brown to dark brown. Aerial mycelium ash-gray. Soluble pigment red-brown.

Glucose-asparagine agar: Growth yellow-brown. Aerial mycelium ash-gray. Soluble pigment yellow-brown.

Glycerol malate agar: Growth dark brown. Aerial mycelium ash-gray to red-gray. Soluble pigment gray to dark brown.

Nutrient agar: Growth dark brown. Aerial mycelium absent or lead-gray. Soluble pigment dark brown. Melanin-positive.

Glucose-peptone agar: Growth dark brown. Aerial mycelium white. Soluble pigment reddish to dark brown.

Starch agar: Growth light yellow to reddish-brown. Aerial mycelium gray-white, later red-gray. Soluble pigment lacking or reddish-brown. Hydrolysis strong.

Potato: Growth brownish-black. Aerial mycelium reddish-white. Soluble pigment dark brown.

Gelatin: Growth dark brown. Aerial mycelium white-gray. Soluble pigment chestnut-brown. Good liquefaction.

Milk: Growth dark brown. Aerial mycelium white, later yellowish-red. Soluble pigment dark brown. Peptonization none or slight.

Cellulose: No growth.

Antagonistic properties: Produces resistomycin, which is active against gram-positive bacteria.

Remarks: Gause *et al.* (1957) have described certain closely related forms, such as *A. griseorubiginosus* with a variety *spiralis*, and *A. variabilis* with a variety *roscolus*.

Type culture: IMRU 3658.

207. *Streptomyces reticuli* (Waksman and Curtis, 1916; Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 118, 1916; Waksman, S. A. Soil Sci. **8:** 143, 1919).

Morphology: Aerial mycelium gives rise to simple verticils. Sporophores straight or spiral-shaped (sinistrorse) on different media. Spores spherical or oval, smooth, 1.0 to 1.4 μ in diameter (Pl. I a).

Sucrose nitrate agar: Growth colorless, with yellowish tinge, becoming brownish. Aerial mycelium thin, cottony, white to ash-gray. No soluble pigment.

Glycerol malate agar: Growth colorless. Aerial mycelium yellowish. No soluble pigment.

Nutrient agar: Growth wrinkled, gray, becoming brownish. No aerial mycelium. Soluble pigment dark brown.

Potato: Growth gray, with black center. Aerial mycelium ash-gray. Soluble pigment black.

Gelatin: Growth gray to brown. Aerial mycelium white. Soluble pigment faint brown to dark brown. Good liquefaction.

Milk: Coagulation rapid; peptonization slow.

Starch: Growth brownish-gray. Hydrolysis.

Cellulose: Scant growth.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Invertase: Positive.

Temperature: Optimum 25°C.

Antagonistic properties: Some strains produce neomycin or a neomycin-like substance. Some strains reduce double bonds in certain steroids.

Habitat: Soil.

Remarks: According to Ettlinger *et al.* (1958), the verticils are both primary and secondary; no spirals were observed. They also report the species to be melanin-negative. One wonders whether they had a typical culture. This culture was later found to

be identical with *S. abikoensum*. Sakagami *et al.* (1958) described a variety *latumcidicus* that produced no aerial mycelium on most media and formed the antibiotic latumcidin.

Type culture: IMRU 3344.

208. *Streptomyces rimosus* Sobin *et al.*, 1950 (Sobin, B. A., Finlay, A. C., and Kane, J. H. U. S. 2,516,080, July 18, 1950; see also Kochi, M., *et al.* Proc. Natl. Acad. Sci. U. S. **38:** 383–391, 1952).

Morphology: Sporophores long, usually straight, occasionally open or closed spirals depending on composition of medium. Spores cylindrical, 0.6 to 0.7 by 0.8 to 1.4 μ. A microscopic study of *S. rimosus* (strain 3558) grown on yeast extract-glucose agar, after 14 days incubation, revealed the following: Aerial hyphae were long and fairly straight, segmenting into chains of even, bead-like spores. Other aerial hyphae were long, tangled, branching, twisting into spirals, also segmented into chains of bead-like spores.

Sucrose nitrate agar: Growth thin, cream-colored, developing slowly at first, later becoming abundant, much folded or lichenoid; reddish-brown to orange. Aerial mycelium appears first over the drier edge of the growth or in the form of thin white patches. When the culture becomes older, a faint bluish zone appears around the edge of the growth. Soluble pigment faint yellowish.

Glucose-asparagine agar: Growth at first cream-colored, becoming brownish to orange-brown with age. Aerial mycelium white. Soluble pigment yellowish to golden.

Yeast-glucose agar: Growth much more rapid than in synthetic media; lichenoid, cream to brownish. Aerial mycelium appears at an early stage of growth, white, later tending to become mouse-gray. Soluble pigment yellowish.

Nutrient agar: Growth poor, cream-colored to yellowish-brown to mouse-gray. Aerial mycelium white or absent. Soluble pigment absent or yellowish.

Starch agar: Growth limited, cream-colored, with deeper brown center. No aerial mycelium. Limited hydrolysis.

Potato: Growth lichenoid, cream-colored to reddish-brown. Aerial mycelium white to gray to dark brown. Soluble pigment yellowish-brown.

Gelatin: Growth cream-colored to brownish. Aerial mycelium white. Slow liquefaction. No soluble pigment, only a faint yellowish coloration of liquefied portion.

Milk: Heavy surface pellicle, cream-colored to yellowish. Aerial mycelium grayish-white. Peptonization, without coagulation.

Cellulose: No growth.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Antagonistic properties: Produces an antibacterial antibiotic, oxytetracycline, and an antifungal agent, rimocidin.

Habitat: Soil.

Remarks: A variety of *S. rimosus* (forma *paromomycinus*) was briefly described (Brit. Pat. 797,568, July 2, 1958). This variety was isolated from a soil in South America. It differs from *S. rimosus* in certain minor cultural properties (somewhat lighter color on agar media) and in poorer utilization of arabinose. Both form dense clusters of spirals on various synthetic media and on glucose-tryptone agar. The variety produces an antibiotic, paromomycin, apparently closely related to the neomycin group.

Type culture: IMRU 3558; ATCC 10,970.

209. *Streptomyces rochei* Berger *et al.*, 1949 (Berger, J., Jampolsky, L. M., and Goldberg, M. W. Arch. Biochem. **22:** 476–478, 1949; Waksman, S. A. and Lechevalier, H. Guide to the classification and identification of the actinomycetes and their antibiotics. The Williams and Wilkins Co., Baltimore, 1953, p. 40).

Morphology: Sporophores straight, 1.5 μ in diameter; often, but not always spirally twisted; spirals usually short and loose with

rarely more than 2 to 3 coils. Spores oval to elliptical, sometimes spherical, 1.2 to 2.8 by 0.8 to 1.5 μ.

Sucrose nitrate agar: Growth thin, colorless, covered with sandy lavender to dark gray aerial mycelium. Reverse of growth light gray, later becoming grayish-yellow. No soluble pigment.

Nutrient agar: Growth cream-colored. Aerial mycelium white. No soluble pigment.

Calcium malate glycerol: Growth good, raised in center. Aerial mycelium gray, buff around the edges, having a fuzzy appearance.

Glucose agar: Growth smooth, yellowish, covered with white to gray aerial mycelium. Yellowish soluble pigment.

Potato: Growth abundant, lichenoid, cream-colored. Aerial mycelium abundant, cottony, white to gray. Color of plug becomes reddish-tan.

Gelatin: Cream-colored ring, covered with white aerial mycelium. Rapid liquefaction. Faint yellow soluble pigment.

Milk: Cream-colored to brownish ring. Coagulation and rapid peptonization.

Starch: Growth brownish. Aerial mycelium mouse-gray. Diastatic action strong.

Production of H_2S: Negative.

Antagonistic properties: On certain complex nitrogenous media, the organism shows a wide range of antimicrobial activity, partly because of borrelidin.

Remarks: Morphologically, the culture resembles *S. albidoflavus*, *S. californicus*, *S. lipmanii*, and certain others, but it is not believed to be identical to any of them. Ettlinger *et al.* (1958) considered this organism as a strain of *S. fradiae*. Okami and Suzuki (1958) could not demonstrate any spirals on several media tested.

Type culture: IMRU 3602; ATCC 10,739.

210. *Streptomyces roseochromogenes* (Jensen, 1931) Waksman and Henrici, 1948 (Jensen, H. L. Proc. Linnean Soc. N. S. Wales **56:** 359, 1931).

Waksman and Curtis (1916) and Waksman (1919) described an organism as *A. roseus* Krainsky. This culture was, in contrast to Krainsky's organism, chromogenic. Jensen (1931) compared it with his own isolates and changed the name *A. roseus* to *roseochromogenes*, because of the fact that the name *roseus* had previously been used by Namyslowsky (1912).

Morphology: Sporophores form numerous open and closed sinistrorse spirals; sometimes 3 to 5 branches issue together from end point of main stem, giving impression of brooms or verticils. Spores spherical, 1.0 to 1.2 by 1.3 to 3.0 μ (Pl. V, 2b).

Sucrose nitrate agar: Growth thin, spreading, colorless to pale yellow. Aerial mycelium pale grayish-rose.

Glucose-asparagine agar: Growth pale yellow. Aerial mycelium white, later becoming rose-cinnamon, with many small white tufts.

Nutrient agar: Growth wrinkled, yellowish-gray, later brown-red. Aerial mycelium white, then rose-gray. Soluble pigment deep brown.

Potato: Growth wrinkled, yellowish-gray to grayish-black. Aerial mycelium absent or white. Soluble pigment black. Melanin-positive.

Gelatin: Colonies small, cream-colored, in bottom of liquefied zone. Soluble pigment brown. Liquefaction medium.

Milk: Coagulation limited; peptonization slow.

Starch media: Growth colorless, spreading. Hydrolysis good.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Antagonistic properties: Active against various bacteria; produces antibiotic roseomycin.

Habitat: Soil.

Remarks: Jensen (1931) obtained, on plating the tufts of white aerial mycelium arising on agar media, a variant with pure white aerial mycelium.

Type culture: ATCC 13,400.

211. *Streptomyces roseocitreus* Kato, 1953 (Kato, H. J. Antibiotics (Japan) **6A:** 143; **6B:** 206–208, 1953).

Morphology: Sporophores produce numerous open and closed spirals of the dextrorse type. Spores oval, 1.2 to 1.5 by 1.6 to 1.8 μ.

Sucrose nitrate agar: Growth pale olive-buff, later changing to deep olive-buff, ivory-yellow, or colonial buff. Aerial mycelium scant, white. Soluble pigment at first faint creamy, later changing to colonial buff.

Glycerol-calcium malate agar: Growth at first transparent with gray to blackish-blue patches, later becoming light yellowish-olive to reed-yellow. Aerial mycelium thin, white, at first having tinge of gray. Soluble pigment yellowish with tinge of green.

Nutrient agar: Growth olive-buff, later changing to deep olive-buff with bluish patches. No aerial mycelium. Soluble pigment brown.

Starch agar: Growth hyaline, cottony, reverse becoming faint bluish. Aerial mycelium white, later becoming livid pink, and finally pale grayish-vinaceous. Enzymatic zone fair to good.

Potato: Growth thick, folded, pale olive-buff, later deep olive to dark olive. Aerial mycelium at first white, later becoming livid pink to vinaceous-buff. Color of plug blackish-brown.

Gelatin: Whitish colonies on surface of tube. Aerial mycelium scant, white. Soluble pigment brown. Liquefaction slow.

Milk: Growth in yellow ring with patches. Soluble pigment yellowish. No coagulation; peptonization slow.

Cellulose: No growth.

Carbon utilization: Utilizes sucrose, L-arabinose, D-sorbitol, salicin, and sodium acetate; not sodium succinate.

Antagonistic properties: Produces antibiotics roseocitrin A and B.

Habitat: Soil.

212. *Streptomyces roseodiastaticus* (Duché, 1934) nov. comb. (Duché, J. Les actinomyces du groupe albus. P. Lechevalier, Paris, p. 329, 1934).

Morphology: Growth consists of fine mycelium 0.5 to 0.7 μ in diameter. Aerial mycelium of larger diameter, but usually less than 1 μ.

Glucose nitrate agar: Growth limited, cream-colored, becoming white with a brownish reverse; on prolonged incubation the culture becomes rose-gray. Soluble pigment brownish.

Glycerol nitrate agar: Growth cream-colored, becoming rose-violet; reverse red.

Asparagine agar: Growth cream-colored, becoming rose-white; reverse of growth reddish-brown. Soluble pigment yellow.

Tyrosine medium: Pigment brownish, later becoming brown.

Gelatin: Liquefied. No soluble pigment. Melanin-negative.

Potato: Growth cream-colored, becoming brownish-white. Soluble pigment brown, only in presence of glycerol.

Milk: Growth limited. Peptonization slow.

Starch: Diastatic action weak.

Remarks: Closely related to *S. halstedii* and considered as a transitional form. According to Ettlinger *et al.* (1958), this organism is related to *S. griseus*.

213. *Streptomyces roseoflavus* Arai, 1951 (Arai, T. J. Antibiotics (Japan) **4:** 215–221, 1951).

Morphology: Sporophores form spirals. Spores oval to oblong, 0.8 to 1.0 by 1.0 to 1.8 μ.

Sucrose nitrate agar: Growth colorless to yellowish. Aerial mycelium powdery, white to yellow-rose.

Glucose-asparagine agar: Growth colorless to yellowish-white. Aerial mycelium rose-colored.

Nutrient agar: Growth much folded, white-gray to golden yellow. Aerial myce-

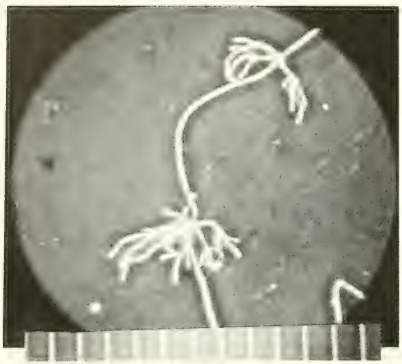

FIGURE 44. Verticil formation by *S. roseoverticillatus* (Reproduced from: Shinobu, R. Mem. Osaka Univ. Ser. B, No. **5**, p. 93, 1956).

lium limited to center of colonies, white to rose.

Starch agar: Growth golden yellow. Aerial mycelium whitish.

Potato: Growth yellow. No aerial mycelium. No soluble pigment.

Gelatin: Liquefaction strong. Colonies at bottom of liquefied zone orange-brown. No soluble pigment. Melanin-negative.

Milk: Ring cream-colored. Coagulation and peptonization rapid, medium becoming strongly alkaline.

Cellulose: Growth on paper fair; cellulose decomposed.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Antagonistic properties: Produces a basic antibiotic, flavomycin, similar to neomycin.

Remarks: Culture similar to *S. microflavus*. Gause *et al.* (1957) described other closely related cultures, such as *A. roseofulvis*.

Type culture: IMRU 3672; ATCC 13,167.

214. *Streptomyces roseoverticillatus* Shinobu, 1956 (Shinobu, R. Mem. Osaka Univ., B (N.S.) **5:** 84–93, 1956).

Morphology: Sporophores produce abundant primary and secondary verticils (Fig. 44). Spores spherical to elliptical, 0.8 to 1.0 μ.

Sucrose nitrate agar: Growth thin, moderate, pinkish-red. Aerial mycelium cottony, pink to pale pink.

Glucose-asparagine agar: Growth red to purple-red. Aerial mycelium cottony, dull red to reddish-brown.

Nutrient agar: Growth reddish-brown to deep brown. Aerial mycelium thin, pinkish to red. Soluble pigment reddish-brown to deep brown.

Potato: Growth brownish-red to dull red. Aerial mycelium powdery, pink to reddish-purple. Soluble pigment brown.

Milk: Growth red. Aerial mycelium pale pink. Coagulation and peptonization strong. Soluble pigment pale brown.

Gelatin: Rapid liquefaction.

Starch: Rapid hydrolysis.

Tyrosinase reaction: Weak.

Cellulose: No growth.

Nitrate reduction: Positive.

Carbon utilization: Utilizes fructose; inositol uncertain. Does not utilize xylose, rhamnose, sucrose, lactose, raffinose, or mannitol.

Habitat: Soil in Japan.

Remarks: Resembles *S. rubrireticuli*.

215. *Streptomyces roseus* (Namyslowsky, 1909; *emend.* Krainsky, 1914; *emend.* Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Namyslowsky, B. Centr. Bakteriol. Parasitenk. Abt. I, Orig. **62:** 564, 1909; Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II, **41:** 682–683, 1914; Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 125, 1916).

Morphology: Sporophores produce numerous open and closed dextrorse spirals. According to Okami, sporophores are straight, without spirals. Spores oval to elongate, 1.5 to 2.0 by 1.1 μ.

Sucrose nitrate agar: Growth colorless. Aerial mycelium pale brownish-vinaceous. No soluble pigment.

Glycerol malate agar: Growth colorless. Aerial mycelium white to rose.

Nutrient agar: Growth white, turning yellowish. No aerial mycelium. No soluble pigment.

Starch agar: Growth colorless. Aerial mycelium white with shade of pink. No soluble pigment. Hydrolysis medium.

Gelatin: Growth yellowish-brown. Aerial mycelium white. Soluble pigment brown. Liquefaction slow; in some cases no liquefaction. Melanin-negative.

Potato: Growth brownish. No aerial mycelium. Soluble pigment brownish or absent.

Milk: No coagulation; gradual peptonization.

Cellulose: No growth.

Invertase: None.

Nitrate reduction: Rapid.

Habitat: Soil.

Remarks: Various cultures have been described under this name. Krassilnikov (1949) considered it as a varietal strain of *S. ruber*.

Type culture: IMRU 3772.

216. *Streptomyces ruber* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41:** 649–688, 1914).

Not *Actinomyces ruber* Ruiz-Cazabo, 1894; not *Actinomyces ruber* (Kruse, 1896) Sanfelice, 1904.

Morphology: Sporophores straight, branching; a few spirals may be formed. Spores spherical and oval, 0.7 to 0.8 by 0.8 to 1.0 μ.

Sucrose nitrate agar: Growth abundant, orange to coral-red. Aerial mycelium red to red-orange to dark red. Pigment insoluble unless vegetable oil present in medium.

Nutrient agar: Growth elevated, wrinkled, olive-green. No aerial mycelium.

Glucose agar: Growth abundant, coral-red.

Potato: No growth.

Gelatin: Growth scant, yellow, flaky. Liquefaction slow, increasing with growth. Melanin-negative.

Milk: Dark ring with red tinge. Coagulation; peptonization gradual.

Starch: Hydrolysis weak.

Sucrose: Inversion positive.

Cellulose: Growth in form of red spots.

Nitrate reduction: Positive, depending on carbon source.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Pigments: Soluble in organic solvents; alcohol extracts a red-violet pigment and petroleum-ether a red-orange pigment (Kriss, 1936).

Antagonistic properties: Strongly effective upon gram-positive bacteria. Various antibiotics are produced by different strains.

Habitat: Soil.

Remarks: Above description is based largely upon that given by Krainsky. Closely related forms include *A. longisporus ruber* Krassilnikov, which is said to form somewhat longer spores, and to give sometimes a brown coloration in protein media. *A. auran-tiogriseus* Gause *et al.* also appears to be closely related.

217. *Streptomyces rubescens* (Jarach, 1931) Umezawa *et al.*, 1952 (Jarach. Boll. sez. ital. soc. intern. microbiol. **3:** 43, 1931; Umezawa, H., Tazaki, T., and Fukuyama, S. J. Antibiotics (Japan) **5:** 469, 1952).

Morphology: Sporophores short, curved, well branched; no spirals. Spores spherical or oval.

Sucrose nitrate agar: Growth at first white, changing to salmon-pink. Aerial mycelium powdery, white. No soluble pigment.

Nutrient agar: Growth same as on sodium nitrate agar.

Blood agar: After 10 days' incubation, growth becomes salmon-pink. Aerial mycelium powdery, white. No soluble pigment. No hemolysis.

Egg media: Growth colorless, changing to coral-pink. Aerial mycelium powdery, white.

Potato: Growth coral-pink. Aerial mycelium powdery, white. Plug changes slightly

to brown. No soluble pigment. Melanin-negative.

Gelatin: Surface growth coral-pink. No liquefaction. No soluble pigment.

Milk: Growth coral-pink. Aerial mycelium powdery, white. No coagulation and no peptonization. Soluble pigment sometimes slightly reddish.

Starch: No hydrolysis.

Nitrate reduction: None.

Production of H_2S: Negative.

Carbon utilization: Glycerol and glucose utilized, but not other carbohydrates.

Antagonistic properties: Produces an antiviral agent, abikoviromycin.

Remarks: *A. griseoruber* of Gause *et al.* (1957) appears to be a closely related form. This organism is considered by R. Gordon as a *Nocardia*, related to *N. asteroides*.

Type culture: IMRU 3655.

218. *Streptomyces rubrireticuli* (Waksman, 1919) Waksman and Henrici, 1948 (Waksman, S. A. Soil Sci. **8:** 146, 1919).

Synonyms: *Actinomyces reticulus-ruber* Waksman, 1919; *A. reticulus* Bergey, 2nd ed., 1925.

Morphology: Sporophores produce both primary and secondary verticils; composition of medium influences structure of sporophores, glucose-asparagine agar favoring spiral formation. Spores oval-shaped, smooth (Pl. I a).

Sucrose nitrate agar: Growth abundant, spreading, usually pink. Aerial mycelium white, later rose to pink.

Glucose-asparagine agar: Entire growth abundant, spreading, rose-red.

Nutrient agar: Growth red, with yellowish margin, becoming red. Soluble pigment dark brown.

Starch agar: Growth white with red tinge. Hydrolysis fair.

Potato: Growth cream-colored, later pink to dark red. Melanin-positive.

Gelatin: Surface growth yellowish-red to pink. Ready liquefaction. Brown pigment.

Milk: Growth abundant. Coagulation and peptonization.

Invertase: Positive.

Cellulose: Growth good.

Nitrate reduction: Rapid.

Production of H_2S: Positive.

Antagonistic properties: Certain strains produce an antibiotic designated as streptin; others produce trichonin.

Habitat: Soil.

Remarks: Numerous cultures that produce a rose to pink substrate growth, a soluble brown pigment in organic media, and both primary and secondary verticils in the sporophores have been described. It is sufficient to mention *A. biverticillatus* by Gause *et al.* (1957).

Type culture: IMRU 3631.

219. *Streptomyces rutgersensis* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 123, 1916; **8:** 152, 1919).

Morphology: Sporophores produce abundant close and open spirals. Spores spherical and oval, 1.0 to 1.2 μ, with tendency to bipolar staining.

Sucrose nitrate agar: Growth thin, colorless, becoming brownish to almost black. Aerial mycelium white, becoming dull gray.

Glucose-asparagine agar: Growth abundant, brown, becoming black with cream-colored margin. No aerial mycelium appears within 15 days.

Nutrient agar: Growth thin, wrinkled, cream-colored.

Starch agar: Growth gray, spreading. Hydrolysis good.

Potato: Growth abundant, much folded. Aerial mycelium white-gray. Melanin-negative.

Gelatin: Growth cream-colored. Liquefaction medium. No soluble pigment.

Milk: Cream-colored ring. Coagulation and slow peptonization.

Cellulose: Growth scant.

Sucrose: Inversion weak.

Nitrate reduction: Good.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Various strains produce xanthomycin-like substances; others produce ruticin.

Remarks: The pigment formed is not soluble. Krassilnikov (1949) considered the organism, quite incorrectly, as a variety of *A. chromogenes*.

Type culture: IMRU 3350.

220. *Streptomyces sahachiroi* Hata *et al.*, 1954 (Hata, T., Koga, F., Sano, Y., Kanamori, K., Matsumae, A., Sugawara, R., Hoshi, T., and Shima, T. J. Antibiotics (Japan) **7A:** 107–112, 1954).

Morphology: Sporophores form numerous closed spirals with a few open spirals. Spores oval or cylindrical, 0.7 to 1.3 by 0.5 to 0.8 μ.

Sucrose nitrate agar: Growth folded, dark orange. Aerial mycelium velvety, white to pale grayish-brown. Soluble pigment yellowish-brown.

Calcium malate agar: Growth thin, cream-colored with orange-yellow reverse. Aerial mycelium thin, white, powdery. Soluble pigment pale orange-yellow.

Nutrient agar: Growth glistening, white-gray. No aerial mycelium. Soluble pigment light brown to yellow.

Starch agar: Growth thin, yellowish-white. Aerial mycelium thin, powdery, pale red-brown. No soluble pigment. Slow hydrolysis.

Potato: Growth wrinkled, pale yellowish-brown. Aerial mycelium thin, white. Soluble pigment absent or faint brown.

Gelatin: Growth limited, white. No aerial mycelium. No soluble pigment.

Milk: Surface growth white to pale yellow. Coagulation; no peptonization. Strongly alkaline. Soluble pink pigment.

Nitrate reduction: Positive.

Carbon utilization: Xylose, arabinose, lactose, trehalose, mannitol, sucrose, salicin, glucose, maltose, mannose, glycerol, dextrin, fructose, starch, galactose, sorbitol utilized. Rhamnose, raffinose, inositol, esculin, dulcitol, inulin, sodium acetate, sodium citrate, sodium succinate not utilized.

Antagonistic properties: Produces antitumor agent carzinophilin.

221. *Streptomyces sampsonii* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Aerial mycelium produces long, straight sporophores, rarely spiral-shaped. Spores cylindrical, 0.8 to 1.0 by 0.5 μ (spores oval to spherical, Waksman and Gordon).

Sucrose nitrate agar: Growth wrinkled, pale gray to white. Aerial mycelium very scant, white. Soluble pigment green to buff.

Potato: Growth wrinkled, grayish. Aerial mycelium white. Soluble pigment golden brown (none, Waksman and Gordon).

Gelatin: Surface growth scant, gray. Aerial mycelium trace, whitish. Liquefaction rapid. Melanin-negative.

Milk: Surface growth good, whitish. No aerial mycelium. No coagulation; no peptonization (rapid peptonization, Waksman and Gordon).

Starch: No hydrolysis (rapid hydrolysis, Waksman and Gordon).

Nitrate reduction: Positive.

Tyrosinase reaction: Negative.

Temperature: 28°C.

Habitat: Potato scab.

Type culture: IMRU 3371.

222. *Streptomyces sayamaensis* Arishima *et al.*, 1955 (Arishima, M., Sekizawa, Y., Sato, T., and Miwa, K. J. Agr. Chem. Soc. Japan **29:** 810–817, 1955).

Morphology: Sporophores straight, spores short rods to cylindrical, 1.0 by 1.5 μ.

Sucrose nitrate agar: Growth pale yellow. Aerial mycelium gray with brownish tinge. Soluble pigment yellow.

Glucose-asparagine agar: Growth white to pale yellow, turning pale brown. Aerial mycelium white, becoming pale brown.

Calcium malate agar: Growth limited.

Aerial mycelium gray. Soluble pigment brownish.

Starch agar: Growth brownish-yellow. Aerial mycelium white, turning gray. Hydrolysis.

Nutrient agar: Growth pale orange-yellow. No aerial mycelium.

Potato: Growth heavy, pinkish-gray with purplish tinge. Soluble pigment reddish-brown.

Gelatin: Surface pellicle pale yellow. No soluble pigment. No liquefaction in 15 days at 26°C. Melanin-negative.

Milk: Yellow-gray ring. Coagulation and peptonization.

Nitrate reduction: Negative.

Cellulose: No growth.

Optimum temperature: 35–37°C.

Tyrosinase reaction: Negative.

Carbon utilization: Utilizes D-galactose, sucrose, maltose, sodium citrate and succinate; does not utilize xylose, arabinose, lactose, rhamnose, raffinose, inulin, mannitol, sorbitol, inositol, and salicin.

Antagonistic properties: Produces chlortetracycline.

Habitat: Soil in Japan.

Remarks: Related to *S. aureofaciens*.

223. *Streptomyces scabies* (Thaxter, 1891) Waksman and Henrici, 1948 (Thaxter, R. Ann. Rept. Conn. Agr. Expt. Sta. 1891, p. 153).

Morphology: Sporophores much branched, wavy or slightly curved; occasionally form spirals. Spores cylindrical, 0.8 to 1.0 by 1.2 to 1.5 μ (Fig. 45).

Sucrose nitrate agar: Growth abundant, wrinkled, raised, gray to cream-colored. Aerial mycelium cottony, white to gray.

Glucose-asparagine agar: Growth restricted, folded, cream-colored. Aerial mycelium scant, white to gray.

Nutrient agar: Growth wrinkled, white to straw-colored, opalescent to opaque. No aerial mycelium. Soluble pigment deep golden brown.

Potato: Growth gray, opalescent, becoming wrinkled, black. Aerial mycelium scant, grayish-white. Color of plug brown.

Gelatin: Surface growth cream-colored, becoming brown. Liquefaction slow. Soluble pigment yellowish.

Milk: Surface ring brown, with greenish tinge. Coagulation and peptonization limited.

Starch: Hydrolysis.

FIGURE 45. Sporophores of *S. scabies*, showing chains of transparent spores, × 15,000 (Courtesy of E. Baldacci, University of Milan, Italy).

Sucrose: Inversion.

Nitrate reduction: Positive.

Tyrosinase reaction: Strong.

Antagonistic properties: Certain strains show positive antimicrobial action; others are negative.

Habitat: Numerous strains of this organism have been isolated from various forms of potato scab and sugar beet scab throughout the world. True causative agent of scab.

Remarks: According to Hoffmann (1958), growth on synthetic agar is reddish with dark gray aerial mycelium; on glucose agar, growth is colorless with blue-gray aerial mycelium; on asparagine agar, growth is dark red with no aerial mycelium.

Closely related forms include *S. clavifer*, *S. spiralis*, *S. carnosus*, and *S. sampsonii* described by Millard and Burr; also *A. xanthostromus* and *A. ochroleucus* of Wollenweber. Various strains differ in the amount of aerial mycelium produced and in their biochemical properties.

Type culture: IMRU 3018.

224. *Streptomyces setonii* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight, wavy, formed in clumps. Spores oval, 0.6 to 0.8 by 0.85 μ.

Sucrose nitrate agar: Growth abundant, smooth, yellow to brown. Aerial mycelium gray to olive-buff. Soluble pigment faint yellowish to brown.

Nutrient agar: Growth colorless. Aerial mycelium smooth, white. Soluble pigment brownish. Melanin-negative.

Glucose agar: Growth lichenoid, gray to brown. Aerial mycelium abundant, white to olive-buff. Soluble pigment golden brown.

Potato: Growth heavy, wrinkled, brown to red-violet. Aerial mycelium abundant, white to green to olive-buff.

Gelatin: Surface growth gray. Aerial mycelium white. Rapid liquefaction. Soluble pigment brownish.

Milk: Surface growth, covered with ring of white aerial mycelium. Questionable coagulation, followed by rapid peptonization.

Starch agar: Growth cream-colored. Aerial mycelium patchy, white. Hydrolysis.

Cellulose: Growth colorless.

Nitrate reduction: Positive.

Temperature: Grows well at 37.5°C.

Habitat: Scabby potatoes.

Remarks: Millard and Burr also described a similar form under the name *A. setonii flavus*. Ettlinger *et al.* (1958) consider this organism as a strain of *S. griseus*. Hoffmann (1958) described an organism with light gray aerial mycelium as a strain of *S. setonii*.

Type culture: IMRU 3375.

225. *Streptomyces somaliensis* (Brumpt, 1906; *emend.* Erikson, 1935) Waksman (Brumpt, E. Arch. Parasitol. Paris **10:** 489, 1906; Precis de Parasitologie. Paris, 2nd ed., p. 967, 1913; Erikson, D. Med. Research Council (Brit.) Spec. Rept. Ser. No. **203:** 1935, p. 17–18).

Morphology: Substrate growth made up of simple branching, unicellular mycelium with long straight filaments. Aerial mycelium forms straight nonsegmented sporophores with typical chains of spores, 1.25 μ in diameter.

Glucose-asparagine agar: Growth thin, smooth, and soft.

Glycerol nitrate agar: Growth abundant, colorless to dark gray and black.

Nutrient agar: Growth abundant, granular, yellowish, with small discrete colonies at margin; later growth colorless, colonies umbilicated.

Potato: Colonies round and oval, partly piled up in rosettes. Aerial mycelium whitish-gray. Plug discolored. Later, aerial mycelium becomes transient, growth nearly black.

Blood agar: Growth in form of small, dark brown colonies. Round and umbilicated, piled up in confluent bands. Reverse red-black. Blood hemolyzed.

Dorset's egg medium: Growth colorless,

becoming opaque, cream-colored, very wrinkled. Later, rough, yellow; medium liquefied.

Gelatin: Growth cream-colored. Black sediment at bottom. Rapid liquefaction.

Milk: Surface pellicle heavy, wrinkled. Milk coagulated and completely peptonized.

Starch: Hydrolysis.

Habitat: Frequently found in Africa.

Remarks: Although *S. somaliensis* has long been known, there has been, until recently, no detailed description of the organism beyond the fact that it possesses around the grain a distinctly hard sheath which is insoluble in potash and eau de javelle. The rare occurrence of septa and occasional intercalary chlamydospores is reported by Brumpt, but has not been confirmed by Erikson. Chalmers and Christopherson merely mentioned the growth on potato as yellowish-white and lichenoid, without describing any aerial mycelium. According to Mariat, *S. somaliensis* hydrolyzes gelatin, serum albumin, and egg albumin; utilizes casein hydrolyzate, but not urea, $(NH_4)_2SO_4$ and KNO_3 as nitrogen sources; utilizes glucose, maltose, and fructose, but not xylose, starch, mannitol, or paraffin as carbon sources.

226. *Streptomyces spectabilis* Dietz, 1957* (Brit. Pat. 811,757, April 8, 1959; Am. Rev. Tuberc. **75:** 576, 1957).

Morphology: Sporophores monopodially branched, long, straight. Pigment granules produced in both substrate and aerial mycelium.

Sucrose nitrate agar: Growth mottled orange to cream-orange. Aerial mycelium mottled orange to orange.

Starch-nitrate agar: Growth cream-colored, flecked with orange. Aerial mycelium pale pink to orange. Starch hydrolyzed.

Starch-peptone-beef extract agar: Growth cream-colored, turning orange. Aerial mycelium deep orange to pale pink. Soluble pigment yellow.

Gelatin: Medium liquefaction. Soluble pigment slightly yellow to dark brown. Melanin-negative.

Milk: Growth orange. Soluble pigment brown. Peptonization varies with strain. Acid formation by some strains.

Carbon utilization: Utilizes various sugars and organic acids, depending on strain. Does not utilize rhamnose, sucrose, inulin, ducitol, D-sorbitol, fumarates, oxalates, or salicylates.

Nitrate reduction: Negative.

Production of H_2S: Positive. Some strains negative.

Antagonistic properties: Produces antibiotic streptovaricin.

Remarks: Closely related to *S. fulvissimus*.

227. *Streptomyces spheroides* Wallick *et al.*, 1955 (Wallick, H., Harris, D. A., Reagan, M. A., Ruger, M., and Woodruff, H. B. Antibiotics Ann. 1955–1956, p. 909–917).

Morphology: Sporophores form spirals, the majority of which are closed and compact; in some areas the spirals appear ball-like. Spores oval, 0.7 to 1.1 by 1.5 to 2.0 μ.

Sucrose nitrate agar: Substrate growth white, becoming straw-colored. Aerial mycelium abundant, white, tinged with cream to olive-gray. No soluble pigment.

Glucose-asparagine agar: Substrate growth pale yellow. Aerial mycelium white, becoming gray. No soluble pigment.

Glucose-peptone agar: Growth moderate, yellow. Aerial mycelium grayish-white. No soluble pigment.

Starch agar: Growth heavy, cream- to straw-colored. Aerial mycelium white.

Potato: Growth slow, scant, white, later becoming heavy, gray. Aerial mycelium gray. Soluble pigment dark brown.

Gelatin: Cream-colored, flaky sediment. Rapid liquefaction. No soluble pigment.

Milk: Slow coagulation and peptonization. Slight acidification.

* Personal communication.

Cellulose: No growth.

Carbon utilization: No gas from adonitol, arabinose, cellobiose, dextrin, dextrose, galactose, lactose, levulose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sucrose, or xylose.

Antagonistic properties: Produces antibiotic novobiocin.

Habitat: Soil.

Remarks: According to Kuroya *et al.* (1958), this organism is related if not identical to *S. griseoflavus*.

228. *Streptomyces spiralis* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight or spiral-shaped. Spores cylindrical, 1.0 to 1.7 by 0.9 μ (Fig. 46).

Sucrose nitrate agar: Growth rough or granular, yellowish-golden. Aerial mycelium vinaceous-buff to dark grayish-olive. Soluble pigment pale vinaceous to fawn-colored.

Potato: Growth poor, wrinkled, grayish-vinaceous. Aerial mycelium white to grayish-vinaceous. Plug colored brown around and below growth.

Gelatin: Growth limited, gray. Aerial mycelium scant, white. Liquefaction rapid. Melanin-negative.

Milk: Surface growth good. Aerial mycelium abundant, white. Coagulation and rapid peptonization.

Starch: No hydrolysis.

Nitrate reduction: None.

Tyrosinase reaction: Negative.

Habitat: Potato scab.

Remarks: Krassilnikov (1949) considered this organism as belonging to the *A. scabies* group.

229. *Streptomyces spiroverticillatus* Shinobu, 1958 (Shinobu, R. Botan. Mag. Tokyo **71:** 87–93, 1958).

Morphology: Verticil formation usually occurs near base of aerial mycelium, but generally not so remarkable as in the other verticil-forming species. Occasionally, very few tufts on the skirt of the colony. Nitella type verticils, generally primary only, seldom secondary. About 2 to 4 short radial branches. On synthetic media, many spirals in form of curled tips with 1 to 2 turns, seldom 3 turns; diameter of spirals about 5 to 8 μ; sometimes snail-like and hook-like curls. Occasionally loose or closed spirals with 2 to 3 verticil turns, sinistrorse. Spores spheroid, somewhat ellipsoid; about 0.8 μ in length (Fig. 47).

Sucrose nitrate agar: Growth colorless to

FIGURE 46. Sporophores of *S. spiralis*, showing that not all are transformed into spores, × 8,000 (Courtesy of E. Baldacci, University of Milan, Italy).

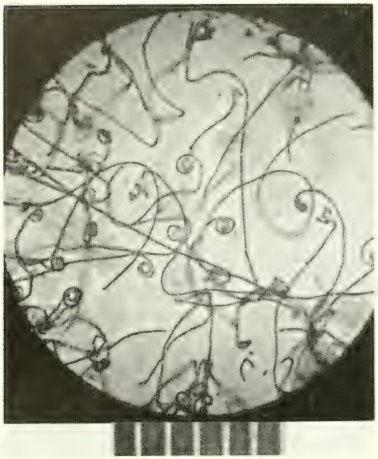

FIGURE 47. *S. spiroverticillatus* (Reproduced from: Shinobu, R. Botan. Mag. (Tokyo) **71:** 88, 1958).

pale brown to yellowish-orange. Aerial mycelium thin, somewhat cottony, white.

Glucose-asparagine agar: Growth pale yellow-orange. Aerial mycelium good, cottony, white to brownish.

Calcium malate agar: Growth yellow-orange to light brown. Aerial mycelium abundant, cottony, white.

Nutrient agar: Growth golden yellow to buff. No aerial mycelium. Soluble pigment pale brown.

Potato: Growth yellowish-brown to brown. Aerial mycelium abundant, cottony, white to brownish-white. Soluble pigment brown.

Milk: Growth yellow to yellow-orange. Aerial mycelium poor, light brownish-gray. Soluble pigment yellowish-orange. No coagulation; rapid peptonization.

Gelatin: Growth poor; liquefaction strong.

Tyrosinase reaction: Somewhat unstable, generally positive, weak.

Diastase reaction: Fairly strong.

Nitrate reduction: Positive.

Carbon utilization: Utilizes lactose, fructose, and xylose. Sucrose and inositol uncertain. Does not utilize rhamnose, mannitol, and raffinose.

Habitat: Soil.

230. *Streptomyces sulphureus* (Rivolta, 1882 *emend.* Gasperini, 1894) Waksman (Rivolta, S. Arch. path. Anat. Phys. **88:** 389, 1882; Gasperini, G. Centr. Bakt. Abt. 1, **15:** 684, 1894; Waksman, S. A. Soil Sci. **8:** 102–104, 1919).

Synonym: *Actinomyces bovis* (Harz) Waksman, 1919.

Not *A. sulphureus* Berestnew, 1897.

This organism is usually found in culture collections under the name of *Actinomyces bovis*. Baldacci (1937, 1947) emphasized the synonymy of this organism, listing as many as 13 different names. The following description is based upon the data of Waksman (1919), who also spoke of it as *A. bovis*.

Sucrose nitrate agar: Growth white, turning yellow. Aerial mycelium light, powdery, sulfur-yellow. No soluble pigment.

Calcium malate-glycerol agar: Growth brownish. No aerial mycelium.

Nutrient agar: Growth at first cream-colored, later becoming fawn-colored, brown, then almost black. Aerial mycelium pale yellow-green. No soluble pigment. Melanin-negative.

Glucose agar: Growth yellowish, later becoming dark. Aerial mycelium thin, sulfur-yellow.

Starch: Fair hydrolysis.

Potato: Growth abundant, much wrinkled, gray to canary-yellow. Aerial mycelium yellow, turning sulfur-yellow. Plug at first not pigmented, later turning brownish.

Gelatin: Growth gray to brownish. No aerial mycelium. No soluble pigment. Liquefaction rapid at 37°C; slow at 18°C.

Milk: Surface growth thin, yellowish. Coagulation and peptonization.

Carbon utilization: Ready utilization of glucose, lactose, sucrose, maltose, glycerol, and various organic acids.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Invertase: None reported.

Remarks: Ettlinger *et al.* (1958) considered certain strains of this organism as belonging to the *S. griseus* series.

231. *Streptomyces tanashiensis* Hata *et al.*, 1952 (Hata, T., Ohki, N., and Higuchi, T. J. Antibiotics (Japan) **5:** 529–534, 1952).

Morphology: Sporophores almost straight. Spores spherical to oval, 1.0 by 1.2 μ.

Sucrose nitrate agar: Growth grayish-yellow. Aerial mycelium white-gray, turning brownish-gray. Soluble pigment light yellow.

Potato: Growth brown. Aerial mycelium dark gray to whitish-gray. Soluble pigment dark brown.

Gelatin: Soluble pigment brown. Rapid liquefaction.

Milk: Yellowish surface ring. Coagulation and peptonization.

Starch: Hydrolysis. Most suitable for antibiotic production.

Nitrate reduction: Negative.

Tyrosinase reaction: Positive.

Production of H_2S: Positive.

Optimum pH: 5.8 to 6.5.

Antagonistic properties: Produces luteomycin.

Habitat: Soil.

Remarks: Resembles *S. aureus* and *S. antibioticus*.

232. *Streptomyces tendae* Ettlinger *et al.*, 1958 (Ettlinger, L., Corbaz, R., and Hütter, R. Arch. Mikrobiol. **31:** 351, 1958).

Morphology: Sporophores form verticils; chains of spores as open, regular spirals. Spores smooth (Pl. I c).

Glycerol nitrate agar: Growth thin, light yellow. No aerial mycelium. No soluble pigment.

Glucose-asparagine agar: Growth light yellow to light carmine. Aerial mycelium cottony, cinnamon-brown.

Calcium malate agar: Growth light yellow to brownish-yellow. Soluble pigment brownish-yellow.

Gelatin: Growth light yellow. Aerial mycelium sparse. Liquefaction limited. Soluble pigment dark brown.

Starch agar: Growth thin, light yellow. Limited hydrolysis of starch.

Potato: Growth brown to dark. Aerial mycelium powdery, chalk-white.

Milk: Growth brownish-yellow. Aerial mycelium sparse. No coagulation; weak peptonization.

Antagonistic properties: Produces antibiotic carbomycin.

Habitat: Soils in France.

Remarks: Organism said to be melanin-negative, although dark brown pigment reported on gelatin.

233. *Streptomyces tenuis* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight. Spores cylindrical, 0.9 by 0.8 μ.

Sucrose nitrate agar: Growth thin, flat, yellowish-drab. Aerial mycelium deep olive-buff. Soluble pigment pale orange-yellow.

Glucose agar: Growth thin, flat. Aerial mycelium olive-buff. Soluble pigment green.

Potato: Growth good. Aerial mycelium deep olive-buff. Soluble pigment gray to olive to black.

Nutrient potato agar: Growth wrinkled, grayish. Aerial mycelium white to vinaceous-fawn. Soluble pigment golden brown.

Gelatin: Growth pale gray. Aerial mycelium scant, white. Rapid liquefaction. Soluble pigment yellow.

Milk: Growth good. Aerial mycelium white. Coagulation; incomplete peptonization.

Starch: Hydrolysis.

Nitrate reduction: Negative.

Tyrosinase reaction: Negative.

Habitat: Potato scab.

234. *Streptomyces thioluteus* Okami, 1952

(Okami, Y. Taxonomic study of antibiotic streptomyces. Thesis, Hokkaido University, Japan, 1952).

Morphology: Aerial hyphae with few branches. No spirals, but verticils produced occasionally, depending on composition of medium.

Glycerol nitrate agar: Growth yellowish-brown, penetrates deep into medium. Aerial mycelium thin, white, with dark yellowish tinge. Soluble pigment yellowish-brown.

Nutrient agar: Growth wrinkled, yellowish-brown. Aerial mycelium scant, white. Soluble pigment slight, yellowish-brown.

Starch agar: Growth thin, cream-colored. No aerial mycelium. No hydrolysis.

Gelatin: Growth yellowish-brown at bottom of liquefied portion. No aerial mycelium. Soluble pigment slight, yellowish-brown. Slow liquefaction.

Potato: Growth wrinkled, cream to yellowish. No aerial mycelium. Soluble pigment slight, yellowish-brown.

Milk: Growth on surface of milk yellowish. Aerial mycelium scant. Coagulation occurs in 2 to 3 days, followed by slow peptonization.

Blood agar: Growth dark gray with greenish tinge. Aerial mycelium dark. No hemolysis.

Nitrate reduction: Negative.

Production of H_2S: Negative.

Antagonistic properties: Produces antifungal substance, aureothricin (Maeda, 1953).

Type culture: ATCC 12,310.

235. *Streptomyces tumuli* (Millard and Beeley, 1927) Waksman (Millard, W. A. and Beeley, F. Ann. Appl. Biol. **14:** 296–311, 1927).

Sucrose nitrate agar: Growth gray, later becoming opaque dark. Aerial mycelium arises at center of growth, at first white, later becoming pale gray. Surface of growth covered with colorless drops leaving small black craters. No soluble pigment.

Glucose-asparagine agar: Growth wrinkled, pale gray. Aerial mycelium white, arising in concentric rings around a dark bare center. Soluble pigment olive-colored.

Nutrient agar: Growth good, lustrous, slimy, gray. No aerial mycelium. No soluble pigment. Melanin-negative.

Potato: Growth heavy, slimy, black. No aerial mycelium. Soluble pigment grayish-brown.

Gelatin: Growth beaded. No aerial mycelium. Liquefaction rapid. No soluble pigment.

Milk: Growth good; no aerial mycelium. Coagulation and slight peptonization.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Habitat: Mound scab of mangels.

236. *Streptomyces venezuelae* Ehrlich *et al.*, 1948 (Ehrlich, J., Gottlieb, D., Burkholder, P. R., Anderson, L. E., and Pridham, T. G. J. Bacteriol. **56:** 467–477, 1948; Pridham, T. G. and Gottlieb, D. J. Bacteriol. **56:** 107–114, 1948).

Morphology: Sporophores monopodially branched, straight or slightly and irregularly curved. Spores oval to oblong, 0.4 to 0.9 by 0.7 to 1.6 μ, smooth (Pl. II p).

Sucrose nitrate agar: Aerial mycelium light lavender.

Nutrient agar: Substrate growth yellow to brown. Aerial mycelium gray. Soluble pigment dark brown to black.

Calcium malate agar: Substrate growth yellow to brown; aerial mycelium gray.

Glucose agar: Soluble pigment dark brown.

Potato: Growth abundant, gray to dark brown. Aerial mycelium gray. Soluble pigment dark brown or black.

Gelatin: Liquefaction rapid. Soluble pigment dark brown.

Milk: Peptonization. Soluble pigment dark brown.

Starch agar: Growth white to lavender. Hydrolysis.

Nitrate reduction: Positive.

Tyrosinase reaction: Positive.

Production of H_2S: Positive.

Carbon utilization: Good growth: xylose, arabinose, rhamnose, D-glucose, D-mannose, D-fructose, D-galactose, cellobiose, starch, dextrin, glycerol, acetate, citrate, succinate, and salicin. Slight or no growth: D-ribose, sucrose, raffinose, inulin, erythritol, dulcitol, mannitol, sorbitol, inositol, and malate. No growth: formate, oxalate, tartrate, salicylate, phenol, *o*-cresol, *m*-cresol, *p*-cresol.

Antagonistic properties: Produces chloramphenicol, an antibiotic active against various gram-positive and gram-negative bacteria, rickettsiae, and psittacosis group.

Habitat: Different soils.

Remarks: This organism is variable, resembling in some respects *S. lavendulae*, although Okami (1956) found it to be markedly different. Krassilnikov described a form under the name *A. rectus*, and a related form, *A. rectus brunneus*, which belong to this group. Gause *et al.* (1957) described a form as *A. venezuelae* var. *spiralis*. *S. phaeochromogenes* var. *chloromyceticus* Okami is identical with *S. venezuelae*.

Morais *et al.* (1958) described a variety of *S. venezuelae* as *roseospori* with a rose-colored rather than lavender aerial mycelium, not producing any antibiotic and not chomogenic on organic media.

Type species: IMRU 3534; ATCC 10,712.

237. *Streptomyces verne* (Waksman and Curtis, 1916) Waksman and Henrici (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 120, 1916; **8:** 156, 1919).

Morphology: According to Jensen (1931), sporophores are long, spiral-shaped. Spores spherical and oval.

Sucrose nitrate agar: Growth abundant, spreading, lichenoid, glossy, yellowish, becoming brownish. Capacity to produce aerial mycelium lost on cultivation.

Glucose-asparagine agar: Growth abundant, lichenoid, center raised, gray with purplish tinge. No aerial mycelium. Soluble pigment faint brown.

Nutrient agar: Colonies small, grayish, with depressed center, becoming wrinkled. No aerial mycelium. No soluble pigment.

Potato: Growth wrinkled, cream-colored, becoming gray. Aerial mycelium absent or scant, white.

Gelatin: Colonies small, cream-colored. No aerial mycelium. Soluble pigment green, a property lost on continued cultivation. Rapid liquefaction.

Milk: Ring pinkish-brown. Coagulation and rapid peptonization.

Starch: Growth scant, restricted, brownish; hydrolysis rapid.

Cellulose: Growth good.

Nitrate reduction: Positive.

Production of H_2S: Negative.

Temperature: Optimum 37°C.

Antagonistic properties: Limited activity against some bacteria.

Remarks: Soluble green pigment produced by freshly isolated cultures; in time, this pigment becomes brown. According to Ettlinger *et al.* (1958), this organism should be regarded as a strain of *S. olivaceus*.

Type Culture: IMRU 3353.

238. *Streptomyces verticillatus* (Kriss, 1938) Waksman (Kriss, A. Mikrobiologiya **7:** 105–111, 1938).

Morphology: Substrate mycelium produced by monopodial branching. Aerial mycelium characterized by primary verticils produced on straight sporophores. The number of verticils at the proximal ends of the primary sterile hyphae is much larger than in the younger portions. Secondary verticils are also produced at the ends of the primary. Spores cylindrical and oblong, 1.0 to 1.7 by 0.8 μ.

Sucrose nitrate agar: Aerial mycelium well developed, velvety, at first white, later dark gray or gray-green.

Nutrient agar: Growth brown. No aerial mycelium. Soluble pigment brown.

Potato: Soluble pigment brown.

Gelatin: Rapid liquefaction.

Milk: Coagulation and peptonization.

Starch: Hydrolysis.

Cellulose: No growth.

Nitrate reduction: Rapid.

Sucrose: Inversion.

Production of H_2S: Positive.

Antagonistic properties: Weak.

Habitat: Rhizosphere of wheat grown in a salinized soil.

Remarks: *A. verticillatus viridans* was described by Krassilnikov (1941) as a substrain of this organism.

239. *Streptomyces violaceoniger* (Waksman and Curtis, 1916) Waksman and Henrici, 1948 (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 111, 1916).

Synonym: *S. violaceus-niger.*

Morphology: The sporogenous hyphae are frequently sterile. Sporophores monopodially branched. Waksman and Curtis (1916) reported no spirals, but Ettlinger *et al.* (1958) found compact spirals. Spores spherical and oval, 1.2 to 1.5 by 1.2 to 2.3 μ, smooth (Pl. II n).

Sucrose nitrate agar: Growth at first dark gray, turning almost black. Aerial mycelium white to gray after the colony is well developed. Soluble pigment at first bluish, later turning almost black.

Potato: Growth at first very slight, but after 48 hours develops into continuous, thick yellowish-gray smear, which later turns brown, with white aerial mycelium covering the growth. Melanin-negative.

Gelatin: Growth gray; no aerial mycelium. Liquefaction rapid. No change in color.

Production of H_2S: Negative.

Antagonistic properties: Produces antibiotic nigericin.

Habitat: Soil.

Remarks: According to Ettlinger *et al.* (1948) the color of the aerial mycelium is carmine-red to cinnamon-brown; with age, the aerial mycelium liquefies and turns black. This organism was believed to belong to the *S. hygroscopicus* group. Nomi (1960) came to similar conclusions.

240. *Streptomyces violaceoruber* (Waksman and Curtis, 1916) Waksman (Waksman, S. A. and Curtis, R. E. Soil Sci. **1:** 110–111, 1916; **8:** 160–163, 1919).

This organism has an interesting history. In the original description of Waksman and Curtis (1916), it was listed in the text (p. 110) as *A. violaceus*, the word "*ruber*" being left out due to poor proof-reading; in the key, however (p. 130), as well as in the following paper by Waksman (1919), in which a complete description was given, it was correctly listed as *A. violaceus-ruber.* The above error was unfortunately repeated in the first and second (p. 374) editions of Bergey's Manual. In the third edition of this manual (1930), Bergey himself changed the name of this organism to *Actinomyces Waksmanii* (p. 489). In the fourth and fifth (p. 867) editions, it was changed to *Actinomyces coelicolor* (Müller) Lieske, and finally in the sixth and seventh editions to *Streptomyces coelicolor* (Müller) Waksman and Henrici. Only the recent studies in which both organisms, *S. coelicolor* Müller and *S. violaceoruber* Waksman and Curtis, were directly compared (Kutzner, 1956; Zähner and Ettlinger, 1957; Kutzner and Waksman, 1959) demonstrated that they are distinctly different.

There are marked physiological and biochemical differences between *S. coelicolor* and *S. violaceoruber.* They particularly include differences in color and morphology of the aerial mycelium, antagonistic properties, and pigment production. *S. coelicolor* is active upon fungi and yeasts, as first shown by Müller; several strains of *S. violaceoruber* produce antibacterial antibiotics, such as actinorhodin, coelicolorin, and mycetin. The pigment of *S. coelicolor* changes to green at an alkaline reaction, that of *S. violaceoruber* to blue. The nature of the pigment has been

studied by Conn (1943) and by Cochrane and Conn (1947).

Various strains closely related to *S. violaceoruber* have been isolated all over the world; some have been listed as varieties, such as *achrous* and *flavus* (Gause *et al.*, 1957). Most of the strains now in the culture collections, designated as *S. coelicolor*, actually belong to *S. violaceoruber*.

Type culture: Waksman and Curtis strain No. 3030, available in the IMRU culture collection.

Synonyms:

A. violaceus Waksman and Curtis (Waksman and Curtis, 1916).

A. violaceus-ruber Waksman and Curtis (Waksman, 1918).

A. waksmanii Bergey (Bergey's Manual, 3rd ed. 1930).

A. coelicolor (Müller) Lieske (Bergey's Manual, 4th and 5th ed., 1934, 1939).

S. coelicolor (Müller) Waksman and Henrici (Bergey's Manual 6th and 7th ed., 1948, 1957).

A. coelicolor (Müller) Krassilnikov (Krassilnikov, 1941).

A. coelicolor Krassilnikov (Gause *et al.*, 1957).

Possible synonym: *A. pluricolor* Berestnew *emend.* Krassilnikov.

Morphology: Aerial mycelium monopodially branched; abundant formation of spirals with 3 to 8 turns, sinistrorse. According to Naganishi and Nomi (1954), two or more sporulating branches may grow from the same spot on the main sporophore. Secondary branches may also be produced. Terminal branches are often arranged in clusters or umbellate forms. Terminal hyphae carry many spirals. Spores spherical to oval, 0.7 to 1.0 by 0.8 to 1.5 μ (Pl. IV Ab). Surface of spores smooth. Asporogenous, nonpigmented strains can be obtained by plating out cultures on carbohydrate-free synthetic media containing nontoxic surface-acting agents (Erikson, 1955b).

Sucrose nitrate agar: Substrate growth abundant, colorless at first, becoming red, then blue to dark blue. Aerial mycelium thin, powdery, white, becoming ash-gray, with a bluish tinge; on some media, light pink to cinnamon; sometimes blue drops can be observed on the surface of the aerial mycelium. Soluble red pigment on acid media, changing to dark blue as medium becomes alkaline.

Glycerol-asparagine agar: Growth good, violet to deep blue. Soluble pigment diffuses through medium.

Glucose-asparagine agar: Growth poor; red pigment does not diffuse readily.

Nutrient agar: Growth white, becoming red with white margin. No soluble brown pigment. Melanin-negative.

Potato: Small, brownish, lichenoid colonies. Aerial mycelium white. Mycelium and plug gradually colored red and blue.

Gelatin: Growth cream-colored, becoming pink or blue. Liquefaction slow.

Milk: Gray surface ring, with red or blue tinge. Coagulation limited; peptonization rapid.

Starch agar: Growth pink. Hydrolysis rapid.

Cellulose: Growth good.

Nitrate reduction: Excellent.

Sucrose: Inversion.

Carbon sources: Utilizes L-xylose, L-arabinose, L-rhamnose, D-fructose, raffinose (some strains only faintly), D-mannitol. None or poor utilization by most strains: sucrose, inulin.

Antagonistic properties: Most strains do not show any strong antagonistic effect; several cultures, which seem to belong or are closely related to *S. violaceoruber*, produce coelicolorin, actinorhodin, streptocyanin, and mycetin.

Habitat: Very common, especially in field soils.

Remarks: Ettlinger *et al.* (1958) considered *S. violaceoruber*, quite incorrectly, as a

strain of *S. fradiae*. Krassilnikov (1949) considered it as a synonym of *S. coelicolor*.

Type culture: IMRU 3030.

241. *Streptomyces violaceus* (Gasperini, 1894, *emend.* Krassilnikov) Waksman (Gasperini, G. Centr. Bakteriol. Parasitenk., Abt. I **15:** 684, 1894; Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, p. 15, 1941).

Morphology: Aerial hyphae long, straight, seldom branching; also short, branched hyphae. Sporophores produce open, sinistrorse spirals with 2 to 3 coils. Spores spherical and oval (Fig. 48).

Agar media: Substrate growth lichenoid, at first red, becoming red-blue, finally purple-violet. Some cultures produce fat droplets in the colony, pigmented red or purple. Aerial mycelium white to gray, produced poorly or not at all; some substrates, like cellulose, paraffin, or fats, favor formation of aerial mycelium. Different pigments

FIGURE 48. Sporophores and spores of *S. violaceus*, grown for 11 days on glucose-asparagine agar (top, hyphae); for 8 days on yeast-glucose agar (bottom left, sporophore); for 4 days on potato agar (bottom right, spores) × 13,500 (Reproduced from: Lechevalier, H. A. and Tikhonienko, A. S. Mikrobiologiya **29:** 43–50, 1960).

are formed in different media and under different conditions of growth. Pigments dissolved in medium do not change with reaction.

Sucrose nitrate agar: Growth dark brown. Aerial mycelium white. Soluble pigment becomes violet to dark violet.

Potato: Growth red-brown to brown. Aerial mycelium, if present, white. Soluble pigment grayish-brown.

Gelatin: Aerial mycelium white. Liquefaction slow. Soluble pigment gray-brown.

Milk: Growth-grayish brown. Coagulation questionable; peptonization slow.

Starch: Hydrolysis weak.

Cellulose: Growth weak, violet. Aerial mycelium light gray.

Nitrate reduction: Positive.

Sucrose: Rapid inversion.

Pigment: According to Kriss (1936), the pigment is soluble in water and in 96 per cent alcohol.

Melanin: According to Hoffmann (1958), this species is melanin-positive.

Antagonistic properties: Exerts strong antagonistic effect upon various gram-positive bacteria.

Remarks: According to Krassilnikov, this species includes *A. violacea* Rossi-Doria, *A. violaceus-caesari* Waksman and Curtis, *Actinomyces* 103 and 109 Lieske; also *A. incanescens* Wollenweber and *A. brasiliensis* Lindenberg (the last is probably a *Nocardia*). A subspecies, *A. violaceus chromogenes* is also included. Some of the cultures described by Gause *et al.* (1957) may also be included here, such as *A. lateritius*, *A. roseoviolaceus*, *A. violaceorectus*, *A. viridoviolaceus*, and *A. violaceus* var. *rubescens*.

Type culture: IMRU 3497.

242. *Streptomyces virgatus* (Krassilnikov) Waksman (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk, SSSR, Moskau, p. 32, 1941).

Morphology: Sporophores short, in form of tufts. Spirals produced rarely. Spores cylindrical, elongated; in some strains round to oval.

Agar media: Substrate growth yellow-green to citron-yellow or pure yellow; on some media pale green. Pigment insoluble. Some strains produce a brown substance in protein media. Aerial mycelium weakly developed, white or pale yellow.

Gelatin: Liquefaction rapid.

Milk: Coagulation and peptonization rapid.

Starch: Hydrolysis rapid.

Cellulose: No growth.

Sucrose: Inversion.

Nitrate reduction: Positive.

Antagonistic properties: None.

Habitat: Soil.

243. *Streptomyces virginiae* Grundy *et al.*, 1952 (Grundy, W. E., Whitman, A. L., Rdzok, E. J., Hanes, M. E., and Sylvester, J. C. Antibiotics & Chemotherapy **2:** 399–408, 1952).

Morphology: Sporophores usually straight; occasionally spirals are observed at or near the tips of the hyphae. Spores cylindrical, 1.1 to 1.5 by 0.75 to 1.0 μ.

Sucrose nitrate agar: Growth sparse, cream-colored. Aerial mycelium light grayish-lavender. No soluble pigment.

Glucose-asparagine agar: Growth sparse, cream-colored to light brown. No aerial mycelium.

Calcium malate agar: Growth abundant, cream-colored. Aerial mycelium white, becoming tinged with grayish-pink to lavender.

Nutrient agar: Growth sparse, white, turning cream-colored to light brown. Aerial mycelium white, turning light grayish-pink to lavender. Soluble pigment light brown.

Oatmeal agar: Growth abundant, cream-colored, turning golden brown. Aerial mycelium abundant, light rose, turning lavender and gray. Soluble pigment pale yellow, turning light brown.

Starch agar: Growth thin, colorless. Aerial mycelium rose to lavender-colored. Hydrolysis.

Potato: Growth abundant, spreading, brownish. Aerial mycelium grayish-pink to lavender. Browning of the potato.

Gelatin: Surface pellicle gray to brownish. Aerial mycelium thin, white. Soluble pigment brown. Liquefaction slow.

Milk: Growth brown. Coagulation none; peptonization slow. Milk becomes dark gray-brown or black.

Nitrate reduction: Limited or absent.

Production of H_2S: Positive.

Carbon utilization: Utilizes glucose, mannose, galactose, maltose, starch, glycerol, sodium acetate, sodium citrate. Does not utilize xylose, lactose, sucrose, mannitol, sorbitol, potassium sodium tartrate.

Antagonistic properties: Produces an antibiotic, actithiazic acid.

Remarks: Various related organisms have been listed. It is sufficient to mention *A. gobitricini*, *A. roseolus*, *A. syringini*, and *A. roseolilacinus*, described by Gause *et al.* (1957).

Type culture: IMRU 3651.

244. *Streptomyces viridans* (Krassilnikov, 1941) (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, Moskau, 1941).

Morphology: Sporophores branched, spiral-shaped. Spores cylindrical.

Glycerol nitrate agar: Growth olive-green with soluble green pigment. Aerial mycelium dark gray, olive-colored, or gray-green, velvety, covering the whole growth.

Nutrient agar: Growth brown-green. Soluble pigment brownish. Melanin-negative.

Potato: Growth brown. Aerial mycelium light gray. Soluble pigment olive-green (Hoffmann, 1958).

Gelatin: Rapid liquefaction.

Milk: No coagulation; rapid peptonization; soluble brown pigment.

Starch: Hydrolysis rapid.

Cellulose: Growth poor.

Nitrate: Reduction to nitrite.

Sucrose: Inversion rapid.

Antagonistic properties: None; some strains are weakly active.

Remarks: Related to *S. intermedius*. Drechsler (1919) described two similar strains, Nos. X and XIV. Gause *et al.* (1957) described a related strain as *A. roseoviridis*.

245. *Streptomyces viridis* (Lombardo-Pellegrino, 1903) Waksman (Lombardo-Pellegrino, P. Riforma med. **39:** 1065–1067, 1903. Summarized by Baldacci, E. Atti ist. botan. "Giovanni Briosi" e lab. crittogam. univ. Pavia (Ser. IV) **11:** 221–223, 1939).

Morphology: Sporophores long or short, straight, undulated; frequently producing broom-shaped clumps. Spores ovoid, 0.7 to 1.4 μ in diameter.

Agar media: Substrate growth on all media at first hyaline, later turning green to dark green. Soluble pigment green. The cultures also grow under anaerobic conditions, but produce no soluble pigment. Aerial mycelium on all media cottony, whitish to grayish.

Potato: Growth dark violet. Aerial mycelium white. Melanin-negative.

Production of H_2S: Positive.

Antagonistic properties: Not reported, or negative.

Habitat: Soil.

Remarks: Baldacci and Comaschi (1956) concluded that the culture described by Krainsky (1914) as *A. griseus* belongs more accurately to the *S. viridis* series. According to Hoffmann (1958), the *A. griseus* Krainsky appears to belong to this group, although he refers to it as *A. griseus* Krassilnikov. It is said to produce broom-shaped sporophores with spirals. Growth colorless, turning light brown. Aerial mycelium velvety, light gray turning dark dray. No soluble pigment. Melanin-negative. Growth on potato lichenoid. Milk not coagulated, but peptonized. Gelatin liquefied. Starch hydrolyzed. It grows on cellulose.

Certain other forms belonging to this group have been described, such as *A. griseus variabilis* and *A. griseus zonatus* of Krassilnikov (1949), *A. badius* and *A. malachiticus* of Gause *et al.* (1957). Krassilnikov also listed *viridis sterilis* as a strain that lost the capacity to produce aerial mycelium.

Millard and Burr (1926) described, under *A. viridis*, an organism that produces dark to black growth on sucrose-nitrate agar, with a mouse-gray aerial mycelium, gradually becoming black. On nutrient agar, growth is at first colorless, gradually becoming gray; aerial mycelium gray to mouse-gray. On gelatin, it produces a thin colorless growth and a faint brownish pigment; rapid liquefaction.

Duché (1934) described an organism under the name *A. viridis*; he later changed this name to *A. baarnensis*. This organism was isolated as a contaminant of cultures of *S. albus* and *S. lavendulae*.

Type culture: IMRU 3372 (strain of Millard and Burr).

246. *Streptomyces viridochromogenes* (Krainsky, 1914) Waksman and Henrici, 1948 (Krainsky, A. Centr. Bakteriol. Parasitenk. Abt. II., **41**: 684–685, 1914).

Morphology: Sporophores monopodially branched, with numerous open or compact, sinistrorse spirals, 3 to 5 μ in diameter, occurring as side branches. Spores short, oval or spherical, 1.25 to 1.5 μ (Figs. 49–51); surface covered with long spines (Pl. II k).

Sucrose nitrate agar: Growth cream-colored with dark center, becoming dark green; reverse yellowish to light cadmium. Aerial mycelium white, becoming light green to light blue.

Glucose-asparagine agar: Growth abundant, spreading, wrinkled, gray, becoming black. Aerial mycelium appears late; white, later becoming green to light blue.

Nutrient agar: Growth restricted, gray, with greenish tinge. No aerial mycelium. Soluble pigment brown.

Potato: Growth abundant, gray-brown.

FIGURE 49. Chains of spores of *S. viridochromogenes*, grown for 16 days on glucose-asparagine-$CaCl_2$ agar, × 13,500 (Reproduced from: Lechevalier, H. A. and Tikhonienko, A. S. Mikrobiologiya **29**: 43–50, 1960).

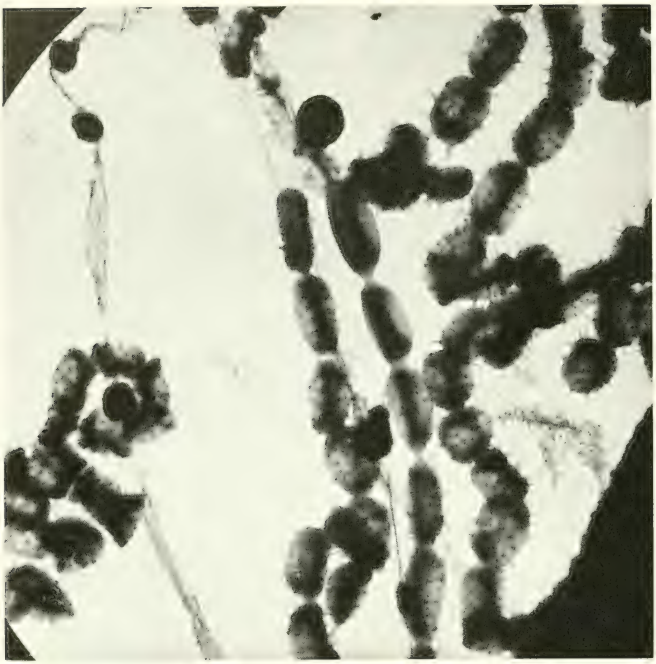

FIGURE 50. *S. viridochromogenes* grown for 30 days on potato agar, × 13,500 (Reproduced from: Lechevalier, H. A. and Tikhonienko, A. S. Mikrobiologiya **29:** 43–50, 1960).

Aerial mycelium white. Soluble pigment black.

Gelatin: Surface growth cream-colored, becoming greenish. Positive liquefaction. Soluble pigment brown.

Milk: Surface growth dark brown; coagulation and peptonization.

Starch agar: Colonies circular, spreading, yellowish. Hydrolysis.

Cellulose: No growth.

Sucrose inversion: Positive.

Nitrate reduction: Positive.

Production of H_2S: Positive.

Tyrosinase reaction: Positive.

Temperature: Optimum 37°C.

Antagonistic properties: Active upon fungi.

Habitat: Soil.

Remarks: This group occurs abundantly in nature. Gause *et al.* (1958) described various forms under different names, such as *A. bicolor*, *A. coeruleofuscus*, *A. coeruleorubidus*, and *A. coerulescens*, and a variety, *longisporus*. According to Ettlinger *et al.* (1958), *S. chartreusis* also belongs to this group.

247. *Streptomyces viridoflavus* Waksman and Taber, 1953 (Waksman, S. A. and Lechevalier, H. A guide to the classification and identification of the actinomycetes and their antibiotics. The Williams & Wilkins Co., Baltimore, 1953, p. 66).

Not *A. viridiflavus* Duché.

Morphology: Sporophores formed in fascicles; tufts, with some curling of tips, are produced on certain media. Tendency to lose

FIGURE 51. Sporophores of *S. viridochromogenes*, × 30,000 (Courtesy of E. Baldacci, University of Milan, Italy).

property of producing aerial mycelium. Submerged sporulating lateral branches form single spores at the tips.

Sucrose nitrate agar: Growth limited, cream-colored to yellowish green. Aerial mycelium usually absent. No soluble pigment.

Glucose nutrient agar: Growth lichenoid, yellowish-brown to olive-brown. Aerial mycelium abundant, later covering the whole

surface of growth with a mat, yellowish to gray. Soluble pigment brownish or absent.

Glucose-asparagine agar: Growth moist, yellow to yellow-green. Aerial mycelium abundant, grayish-yellow to sulfur-yellow, later overgrown by white sporulating hyphae. Soluble pigment absent or faint yellow.

Nutrient agar: Growth moist, gray to light green with green to almost bluish tinge at bottom of slant. Nonsporulating aerial mycelium appears much later; it is white to gray. No soluble pigment.

Potato: Growth lichenoid, brownish to greenish-yellow to dark olive-green. Aerial mycelium absent, or formed as thin, yellowish layer on drier portions of growth. Soluble pigment absent or dark brown.

Gelatin: Growth in form of surface ring, canary-yellow. Slight liquefaction. Soluble pigment brown to dark brown, a property that may be lost on cultivation.

Starch: Hydrolysis.

Cellulose: Limited growth, no destruction of cellulose.

Production of H_2S: Negative.

Carbon utilization: No growth with sucrose, lactose, or rhamnose; good growth on mannose and glucose.

Antagonistic properties: Produces an antifungal substance, candidin.

Habitat: Soil.

Type culture: IMRU 3685.

248. *Streptomyces viridogenes* (Millard and Burr, 1926) Waksman (*S. viridis* of Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores long, sympodially branched, straight. Spores spherical, 0.9 μ, smooth (Pl. II o).

Sucrose nitrate agar: Growth abundant. Aerial mycelium olive-gray. Soluble pigment greenish-yellow to blackish-green.

Glucose-asparagine agar: Growth smooth, raised, olive-buff. Aerial mycelium abundant, light gray to deep mouse-gray. Soluble pigment yellowish to greenish-yellow.

Nutrient agar: Growth lichenoid, cream-colored. No aerial mycelium. No soluble pigment.

Nutrient agar with glucose: Growth gray to black. Aerial mycelium gray. Soluble pigment dark brown.

Starch agar: Growth gray to brown. Aerial mycelium thin, white. Hydrolysis positive.

Potato: Growth gray to olive-gray. Aerial mycelium either absent or white, turning gray. Soluble pigment brown.

Gelatin: Growth grayish. Aerial mycelium scant, white to gray. Liquefaction rapid. Soluble pigment light golden brown.

Milk: Surface growth good. Aerial mycelium scant, white. Coagulation rapid and peptonization gradual.

Nitrate reduction: Positive.

Tyrosinase reaction: Negative.

Temperature: Grows well at 37.5°C.

Habitat: Potato scab.

Remarks: Ettlinger *et al.* (1958) reported that this species is melanin-negative.

249. *Streptomyces wedmorensis* (Millard and Burr, 1926) Waksman (Millard, W. A. and Burr, S. Ann. Appl. Biol. **13:** 580, 1926).

Morphology: Sporophores straight, branched. Spores oblong, 0.8 to 0.9 by 0.6 to 0.8 μ.

Sucrose nitrate agar: Growth flat, thin, grayish. Aerial mycelium white to gray.

Nutrient potato agar: Growth wrinkled, grayish. No aerial mycelium. Melanin-negative.

Potato: Growth wrinkled, grayish. Aerial mycelium white. Plug pigmented drab.

Gelatin: Growth fair. No aerial mycelium. Liquefaction medium.

Milk: Growth greenish. Coagulation and slow peptonization.

Starch: Hydrolysis.

Nitrate reduction: Positive.

Tyrosinase reaction: Negative.

Temperature: Grows well at 37.5°C.

Habitat: Potato tubers.

250. *Streptomyces willmorei* (Erikson 1935) Waksman and Henrici, 1948. (Erikson, D. Med. Research Council (Brit.) Spec. Rept. Ser. No. **203:** 19–20, 1935).

Morphology: Submerged growth consists of unicellular mycelium frequently branched at short intervals, presenting peculiar clubbed and budding forms with occasional separate, round, swollen cells. The filaments are characteristically long, homogeneous, and much interwoven. Aerial mycelium profuse in most media, with a marked tendency to produce loose spirals with chains of ellipsoidal spores. Thick aerial clusters may also be formed.

Glucose-asparagine agar: Growth colorless, wrinkled, confluent, with smooth entire margin; large discrete colonies like flat rosettes. Aerial mycelium scant, white.

Glycerol nitrate agar: Round, smooth, cream-colored colonies, heavy texture, margin submerged. Stiff, sparse aerial spikes.

Peptone-beef extract or nutrient agar: Growth heavy, colorless, lichenoid, rounded elevation covered with white aerial mycelium. Later, submerged margin, round confluent growth; aerial mycelium marked in concentric zones.

Potato agar: Fair growth, partly submerged. Aerial mycelium grayish-white.

Gelatin: Colonies minute, colorless. Positive liquefaction.

Milk: Coagulation and slow peptonization.

Dorset's egg medium: Large, round, colorless, scale-like colonies, radially wrinkled, later growth brownish; medium discolored.

Serum agar: Smooth colorless discoid colonies; marked umbilication after 2 weeks.

Production of H_2S: Negative.

Antagonistic properties: Positive.

Source: Streptothricosis of liver.

Remarks: Ettlinger *et al.* (1958) place this species in the *S. griseus* series.

Type culture: IMRU 3332.

251. *Streptomyces xanthophaeus* Lindenbein, 1952 (Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952).

Morphology: No description.

Glycerol nitrate agar: Growth brownish. Aerial mycelium white-gray or reddish-gray. Soluble pigment yellow-brown.

Glycerol malate agar: Growth deep orange. Aerial mycelium white-gray to red-gray. Soluble pigment deep orange.

Glucose-asparagine agar: Growth diffuse, light yellow. Aerial mycelium white. Soluble pigment light yellow.

Nutrient agar: Growth light brown. Aerial mycelium ash-gray to white. Soluble pigment yellow to yellow-brown. Melanin-negative.

Glucose-peptone agar: Growth light yellow. Aerial mycelium ash-gray. Soluble pigment yellow.

Starch agar: Growth lichenoid. Aerial mycelium violet-gray. Hydrolysis rapid.

Potato: Growth lichenoid. Aerial mycelium gray. No soluble pigment.

Gelatin: Growth brown. Aerial mycelium ash-gray. Soluble pigment yellow-brown. Liquefaction strong.

Milk: Growth lichenoid. Aerial mycelium gray to violet. Soluble pigment dark brown. Peptonization strong.

Cellulose: No growth.

Antagonistic properties: Produces geomycin, active against gram-negative bacteria.

Habitat: Limestone deposit in Germany.

Remarks: Related to *S. erythraeus* and *S. erythrochromogenes*. Kutzner (1956) studied five soil isolates. Four strains did not form any spirals; one did. The spores were smooth. The soluble pigment on glucose-peptone agar was dark brown. He thus considered this species as melanin-positive.

Addendum

After the text of this volume was completed, the following newly described forms appeared in print:

Streptomyces aerocolonigenes Shinobu and

Kawato (Botan. Mag. Tokyo **73:** 212–216, 1960).

Actinomyces aureoverticillatus Krassilnikov and Dzi-Shen (Mikrobiologiya **29:** 482–489, 1960).

Streptomyces herbaricolor Kawato and Shinobu (Mem. Osaka Univ. B **8:** 114–119, 1959).

Streptomyces massasporeus Shinobu and Kawato (Botan. Mag. Tokyo **72:** 853–854, 1959).

Streptomyces ostreogriseus (Antibiotic E-129) Brit. Pat. 799,053, July 30, 1958.

Streptomyces psammoticus Virgilio and Hengeller (Farmaco, Ed. Sci. **15:** 164–174, 1960).

Chapter 9

The Genus *Micromonospora*

The genus *Micromonospora* is characterized by the production in nutrient media of a well developed substrate mycelium, 0.2 to 0.6 μ in diameter, partly penetrating into the medium. The substrate or vegetative hyphae are straight or curved, branching, without cross walls. Aerial mycelium is not formed at all or only in a rudimentary, nonsporulating form, when the hyphae arise upward directly from the substrate mycelium.

Multiplication occurs by means of fragments of mycelium and special spores formed singly. A swelling takes place at the end of the sporophore; later the swelling is separated by a cross wall, giving rise to spherical, oval, or oblong spores, 1.0 to 1.5 by 0.8 to 1.2 μ. The sporophores are often branched, each branch forming a spore at the end, giving rise to a grape-like bunch of spores. These germinate in a manner similar to the spores of *Streptomyces*. The mycelium and spores are gram-positive, not acid-fast (Fig. 52).

The colonies of *Micromonospora* are similar to those of *Streptomyces*. They are compact, leathery, smooth or lichenoid, raised or flat. They are frequently colored red or orange or yellow, occasionally brown or green to almost black or blue. The pigments, except the dark brown, are not dissolved into the medium.

In characterizing species of *Micromonospora*, T'ao Ho and Potter (1960) considered morphological properties as primary criteria for identification of the organisms. The important physiological characteristics included the disintegration of cellulose, inversion of sucrose, and the reduction of nitrate. The investigators emphasized that the color of the growth and the form of the colony could not serve as basic characteristics. Certain strains may show different colors for the mass of growth and for the spores. Reproducibility of colony color for a given organism could not be obtained on the same medium. The color itself was not consistent, varying through every shade of yellow, orange, pink, red, and brown. Many species gave more than one colonial form. The large spores of *M. globosa* were very helpful in differentiating it from *M. fusca* or *M. chalcea*.

Micromonospora species are aerobic or anaerobic, and mesophilic. They grow readily at 25–40°C. Thermal death point of the mycelium is 70°C in 2 to 5 minutes; spores resist 80°C for 1 to 5 minutes. They utilize various carbon and nitrogen sources, both organic and inorganic (Fig. 53).

The type species is *Micromonospora chalcea* (Foulerton) Ørskov.

The genus *Micromonospora* comprises nine species, which can be classified as follows:

Classification of the genus *Micromonospora*

A. Aerobic.
  1. Sporophores long.
    1. Sporophores showing little branching.
      a. No aerial mycelium.
        2. *Micromonospora chalcea*
      b. Rudimentary aerial mycelium.
        6. *Micromonospora gallica*

2. Sporophores form extensive branching.
   a. Growth colorless; brown spores appear in mass.
      7. *Micromonospora globosa*
   b. Growth pigmented.
      a¹. Growth green to dark green.
         a². Spores blue.
            3. *Micromonospora coerulea*
         b². Spores black or brown.
            1. *Micromonospora bicolor*
      b¹. Growth pink to orange-colored.
         a². Pigment not excreted into substrate.
            8. *Micromonospora parva*
         b². Red-brown pigment excreted into substrate.
            5. *Micromonospora fusca*

II. Sporophores short.
   1. Growth brown; spores dark brown.
      4. *Micromonospora elongata*

B. Anaerobic.
   9. *Micromonospora propionica*

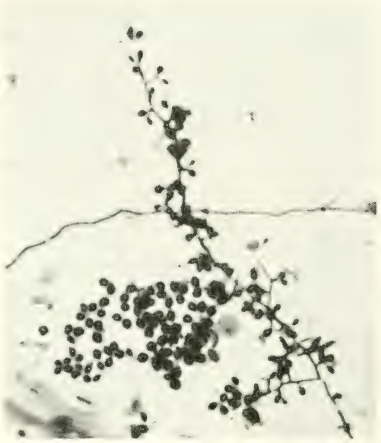

FIGURE 53. *Micromonospora* (double spores) growing in a compost.

Various other micromonosporas have been observed in natural substrates, but either have not been isolated or only insufficiently studied. This is true, for example, of the cellulose-decomposing, facultative anaerobic form studied by Meyer, 1934 (Prevot, 1955); and of *M. cabaelli* Maquer and Comby (Prevot, 1955). It is also true of some of the forms reported by Waksman *et al.* (1939). Some of the micromonosporas (*M. monospora* and *M. vulgaris*) have been placed, because of their temperature optima, among the thermophilic forms.

FIGURE 52. *Micromonospora* (clumps of spores) growing in a compost.

Description of Micromonospora Species

1. *Micromonospora bicolor* Krassilnikov, 1941 (Krassilnikov, N. A. Actinomycetales. Izvest. Akad. Nauk. SSSR, 1941, p. 131).

Morphology: Sporophores long, branching, 10 to 25 μ; spores oval, 1.0 to 1.2 by 0.8 μ.

Synthetic agar: Growth green, smooth; covered with a dark brown to black hue of spore-bearing hyphae. Pigment insoluble.

Nutrient agar: No growth.

Potato: No growth.

Gelatin: No growth. No liquefaction.

Milk: Unchanged.

Starch: Not liquefied.

Sucrose: Inverted.

Cellulose: Good growth and decomposition.

Carbon sources: Glucose, sucrose, levulose, acetic and citric acids.

Nitrogen sources: Ammonium salts and nitrates.

Habitat: Soil.

2. *Micromonospora chalcea* (Foulerton, 1905) Ørskov, 1923 (Foulerton, A. Lancet *1:* 1200, 1905; Ørskov, J. Investigations into the morphology of the ray fungi. Levin and Munksgaard, Copenhagen, 1923).

Morphology: Grows well on all media, especially on glucose-asparagine agar. Growth heavy, compact, raised, pale pink to deep orange, not spreading much into the medium. Hyphae long, thin, branching, nonseptate. Surface of growth smooth or folded, dull or shining. Spore layer well developed, moist and glistening, brownish-black to greenish-black; color sometimes spreading through the whole mass of growth. Spores oval or spherical, formed individually on relatively nonbranching sporophores (Fig. 54).

Gelatin: Liquefaction positive. No soluble pigment.

Milk: Coagulation and peptonization positive.

Starch: Hydrolyzed.

Cellulose: Rapid decomposition.

Chitin: Decomposed.

Nitrate reduction: Positive.

Sucrose: Inverted.

Proteolytic action: Strong.

Temperature: Optimum for growth, 30–35°C. Thermal death point of mycelium, 70°C in 2 to 5 minutes. Spores resist 80°C for 1 to 5 minutes.

Source: Soil, lake mud, and other substrates.

3. *Micromonospora coerulea* Jensen, 1932 (Jensen, H. Proc. Linnean Soc. N.S. Wales **57:** 173, 1932).

Morphology: Growth smooth, lustrous, greenish-blue; pigmentation only on free admission of oxygen. Pigment insoluble. Surface of colonies hard and glossy; thin, white veil on surface resembles aerial mycelium. Spherical blue spores produced on branching short sporophores.

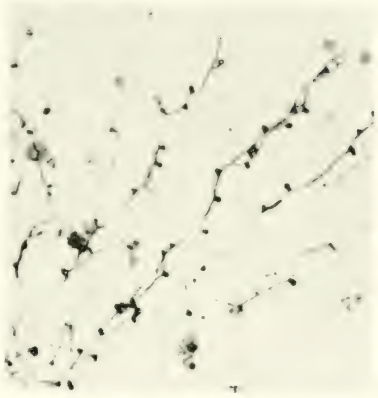

FIGURE 54. *Micromonospora* (single spores) growing in a compost.

Nutrient media: Slow growth.

Liquid media: Growth at bottom, in the form of firm, round, white to pink granules.

Gelatin liquefaction: Rapid.

Milk: Positive coagulation, but very slight peptonization.

Starch: Hydrolyzed.

Cellulose: Not decomposed.

Nitrate reduction: None.

Sucrose: Not inverted.

Source: Occurs rarely in soil.

4. *Micromonospora elongata* Krassilnikov, 1941 (Krassilnikov, N. A. Actinomycetales. Izvest. Acad. Nauk. SSSR, 1941, p. 130).

Morphology: Sporophores short (2 to 3 μ), little branched. Spores oval, 1.0 to 1.3 by 0.8 μ (Fig. 55).

Agar media: Growth poor, adhering to substrate in form of minute pale yellow smooth colonies. Surface is dark brown.

Potato, gelatin, and milk: No growth.

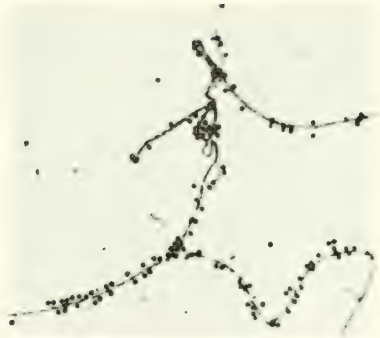

FIGURE 55. *Micromonospora* (single spores) growing in a compost.

Cellulose: Good growth and decomposition.

Sucrose: Inverted.

Nitrate reduction: Positive.

Habitat: Soil.

5. *Micromonospora fusca* Jensen, 1932 (Jensen, H., Proc. Linnean Soc. N.S. Wales, **57:** 178, 1932).

Morphology: Growth heavy, compact, orange-colored, rapidly changing to deep brown and nearly black. Spore layer moist, glistening, grayish to brownish-black. Spores oval or spherical.

Gelatin: Liquefaction slow. Soluble pigment very slight.

Milk: No coagulation, slow peptonization; grayish-brown discoloration of milk.

Starch: Hydrolyzed.

Cellulose: Limited decomposition.

Nitrate reduction: Positive or negative.

Sucrose: Inverted.

Antagonistic properties: Produces the antibiotic micromonosporin.

Source: Soil.

6. *Micromonospora gallica* (Erikson, 1935) Waksman (Erikson, D. Med. Res. Council, London, Pub. No. 4582, 1935, p. 24).

Morphology: Aerial hyphae retarded and rudimentary. Typical single spores produced.

Sucrose nitrate agar: No growth.

Glycerol agar: No growth.

Glucose agar: No growth.

Potato agar: Growth pale pink, moist, granular.

Potato: Growth slow in form of pink, translucent colonies, tending to become umbilicated and heaped up.

Egg media: Colonies minute, becoming confluent, tangerine-colored.

Blood agar: Colonies minute, discrete, pink. No hemolysis.

Gelatin: Growth scant, irregular, pink. Liquefaction slow.

Broth: Pinkish flakes. Small rounded red granules at bottom.

Milk: Surface ring yellowish-pink. Positive coagulation and peptonization.

Habitat: Isolated from blood culture.

7. *Micromonospora globosa* Krassilnikov, 1939 (Krassilnikov, N. A. Mikrobiologiya **8:** 179, 1939; Actinomycetes. Izvest. Akad. Nauk, SSSR, Moskau, 1941, p. 129).

Morphology: Spores spherical, 1.0 μ, arranged in clusters on long branching sporophores.

Agar media: Growth at first colorless, leathery, lichenoid; covered with a dark brownish tarnish of spores.

Gelatin: Liquefaction slight.

Milk: Coagulated and peptonized.

Starch: Hydrolyzed.

Cellulose: Satisfactory growth.

Nitrate reduction: Positive.

Sucrose: Inverted.

Source: Soil.

8. *Micromonospora parva* Jensen, 1932 (Jensen, H. Proc. Linnean Soc. N.S. Wales **57:** 177, 1932).

Morphology: Growth pale pink to orange, compact. Substrate mycelium thin, spreading widely into the agar. Sporulation scant, giving rise to thin, grayish, moist crusts on the surface. Spores oval; in mass gray-colored.

Gelatin: Liquefied slowly.

Milk: Unchanged; or coagulated and slowly peptonized with faintly acid reaction.

Starch: Hydrolyzed.

Cellulose: No decomposition.

Nitrate reduction: None.

Sucrose: Not inverted.

Source: Soil.

9. *Micromonospora propionica* Hungate, 1946 (Hungate, R. E. J. Bacteriol. **51:** 51-56, 1946).

Morphology: Grows very slowly. White spherical colonies produced on cellulose. Colony consists of gradually expanding hollow shell, its outer surface consisting of substrate hyphae, the adjacent inner portion containing numerous spores, and the center relatively devoid of protoplasm.

Media: Highly complex media required.

Cellulose: Good growth. Good decomposition.

Oxygen demand: Obligate anaerobe.

Carbon sources: Glucose and cellulose are utilized.

Fermentation products: Carbon dioxide, acetic and propionic acids.

Temperature: 30–40°C.

Habitat: Gut of termites, rumen of cattle.

Remarks: Prévot (1957) considers this species as an anaerobic *Actinomyces*, which he includes in the genus *Actinobacterium*.

Chapter 10

The Genus *Waksmania* (*Microbispora*)

The genus *Waksmania* (*Microbispora*) is characterized by the paired spores produced on aerial hyphae (Fig. 56).

The fine mycelium (about 1 μ in diameter) is differentiated into (a) primary or substrate mycelium which grows into, and forms a compact layer on top of agar media, and (b) secondary or aerial mycelium which arises from the primary mycelium but grows into the air, away from the agar surface. The substrate mycelium does not bear spores of any type; the aerial mycelium bears spores which are formed in longitudinal pairs. The spores are produced either directly on the aerial hyphae or on sporophores which branch from the aerial hyphae.

The sporophores may be so short that the spores appear to be produced directly on the mycelium. The aerial mycelium forms a bud at the side, and later the bud, or occasionally the tip of the side branch, swells and is separated by a cross wall giving rise to two spherical or oval conidia, 1.4 to 1.6 μ in diameter (Fig. 57).

The germination of the spores and the structure of substrate mycelium are similar to those of *Streptomyces*.

The type species is *Waksmania rosea* Lechevalier and Lechevalier.

Waksmania rosea Lechevalier and Lechevalier, 1957 (Lechevalier, M. P. and Lechevalier, H. J. Gen. Microbiol. **17:** 104–111, 1957).

Synonym: *Microbispora rosea* Nonomura and Ohara, 1957 (Nonomura, H. and Ohara, Y., J. Ferm. Technol., **35:** 307–311, 1957).

Morphology: The dominant form of this organism on some media, after 14 days at 30°C, may consist of chlamydospores. Hyphae do not segment, even in old cultures. "Fairy rings," or alternating areas of aerial mycelium with zones which have none, occur on some media. "Coremia-like" aggregations of aerial hyphae are formed on a variety of substrates. Small branches are produced monopodially in respect to the main axis of the aerial sporogenous hyphae. Spores are borne at the tip of these branches or sporophores. Spores are spherical, 1.5 to 2.0 μ, usually about 1.7 to 1.8 μ. Spores are borne terminally on sporophores, as well as at the tips of the main sporogenous hyphae and branches. They are also borne directly on the sporogenous hyphae. Spores are formed in pairs and, when mature, are very easily detached from the sporophore and from each other.

Agar media: Growth slow on nutrient agar. Color of substrate growth pale pink to coral-orange, changing to chestnut-brown. Diffusible pigment very slight. Aerial mycelium white, powdery, with tendency to form fairy rings on some media.

Sucrose nitrate agar: Growth thin, yellowish-white. Aerial mycelium scant. Some malformed spores appear.

Glucose-asparagine agar: Growth meager, colorless. No aerial mycelium.

Yeast-glucose agar: Growth white-tan, glistening, becoming dark brown and convoluted. No aerial mycelium.

Oatmeal agar: Orange-pink vegetative growth. Aerial mycelium white, with light pink spores. Earthy odor.

Potato: Growth dark reddish-brown. Aerial mycelium has a slight trace of white.

Gelatin: Growth on the bottom of tube white, fluffy. Liquefaction slight.

Milk: Growth on the bottom of the tube white. Surface colonies orange-pink, attached to sides of test tube. Coagulation none; peptonization complete after 1 month. No change in pH.

Starch: Not hydrolyzed.

Sucrose: Not inverted.

Cellulose: Attacked to a very limited degree.

Nitrate reduction: Negative.

Temperature: The organism is a mesophile, growing well at 25–35°C. It produces only sparse growth at 40°C, and does not grow at all at 55°C.

Antagonistic properties: None.

Habitat: Soil. A culture of this organism causing pericarditis and pleuritis has been recently isolated by Louria and Gordon (1960).

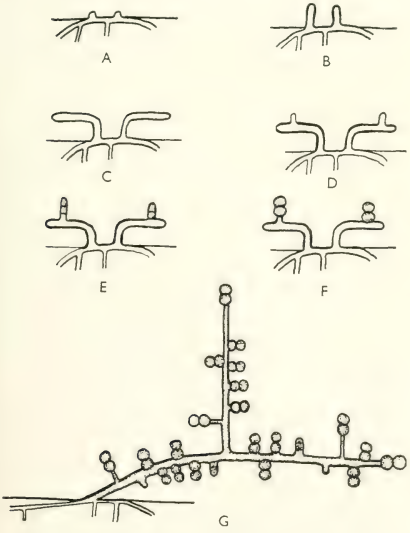

FIGURE 56. *Waksmania rosea*, schematic representation of the formation of aerial mycelium and spores (Reproduced from: Lechevalier, M. P. and Lechevalier, H. J. Gen. Microbiol. **17**:108, 1957).

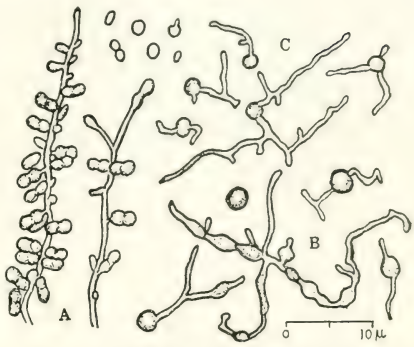

FIGURE 57. *Waksmania* (*Microbispora*) *rosea:* A. sporulation; B. chlamydospores; C. germination of conidia (Reproduced from: Nonomura, H. and Ohara, Y. J. Fermentation Technol. **35**:307, 1957).

Remarks: Thiamine and biotin are essential for growth; biotin also controls pigmentation. Ammonium compounds, nitrates, and urea are not utilized as sources of nitrogen. Asparagine, glutamic acid, and peptone are good sources.

Type cultures: IMRU 3748, 3757.

Nonomura and Ohara (1960a) found the genus *Waksmania* (*Microbispora*) widely distributed in soils of Japan. When a particle of soil was placed on soil-extract agar in a Petri dish, colonies of this group of organisms appeared around the soil particle after a few weeks incubation at 30°C. Five species and two varieties were recognized, including *M. amethystogenes*, *M. amethystogenes* var. *nonreducans*, *M. parva*, *M. chromogenes*, *M. diastatica*, and *M. rosea* var. *nonnitritogenes*.

A system of classification of these species and varieties was proposed, based upon their growth on different media, nitrate reduction to nitrite, production of soluble pigment, and hydrolysis of starch. Some of the strains produced violet crystals, insoluble in water but soluble in benzene. Thiamine was required for growth of all strains, biotin to a limited extent.

Chapter 11

Thermophilic Actinomycetes

Our knowledge of the thermophilic actinomycetes dates back to the early beginnings of general microbiology. The first students of the microbiological population of soils and composts observed that some of the organisms found among the bacteria, actinomycetes, and fungi were able to grow at much higher temperatures than the great majority of the members of these groups.

Globig (1888) was the first to isolate from the soil thermophilic actinomycetes, capable of growing at 52–65°C. Rabinowitsch (1895) and Tsiklinsky (1903) isolated similar cultures from manure, and Noack (1912) isolated them from hay. Numerous other isolations of thermophilic actinomycetes were made, from ordinary soil by Gilbert (1904), from desert sand, feces, air, and peat, as well as from human intestines and from sewage. It has also been observed repeatedly that composted manure, when it has attained a high temperature, or hay which has been allowed to heat in composts, becomes covered with small white patches of fungus-like growth. Miehe (1907) remarked that the appearance of these patches is similar to a coat of lime and is due to actinomycetes. Similar observations of thermophilic composts have been made by various other investigators.

Tsiklinsky inoculated potato slices with soil or with manure and incubated them at 53–55°C. Isolations were made on agar plates after 16 hours. Two cultures were thus obtained. One produced chains of spores and was considered to be, therefore, a true actinomycete of the type now designated as *Streptomyces*. The other formed round or ovoid spores at the end of side branches, by the swelling of the tips. This organism was believed to be widely distributed in nature and was named *Thermoactinomyces vulgaris*. Because of its manner of spore formation, this form was believed to belong to the group of actinomycetes designated by Ørskov (1923) as *Micromonospora*, and was, therefore, classified by Waksman as *Micromonospora vulgaris*. It grew at 48–68°C, with an optimum at 57°C, and no growth at 70°C. It remained inert for a month at 37°C or at lower temperatures, but it became active within 24 hours at 56–57°C. The spores were said not to be destroyed at 100°C, even after 20 minutes. The organism grew readily on most ordinary media; it was proteolytic but not amylolytic. The *Streptomyces*, on the other hand, was weakly proteolytic, and the spores were less resistant to heat (Fig. 58).

Gilbert cultivated from various soils several strains of a thermophilic actinomycete, which he designated as *A. thermophilus*. Growth on potato was much folded, white, later becoming gray on the surface; the plug was darkened by some cultures. The optimum temperature was 55°C; no growth took place at 60°C. Most strains ceased to grow at 45°C, whereas some could be adapted to grow on agar media at 37°C and even at 22°C. The colonies on agar were, after 24 to 48 hours, small, folded, light yellow with a dark-colored center. Gelatin was only slowly liquefied.

Miehe considered hot composts and not soils as the natural substrates of actino-

mycetes. The spores of these organisms were found to lose their vitality rapidly, especially on agar media, but they survived on hay particles. One culture was designated as *A. thermophilus* Berestnew; it grew best at 40–50°C, more slowly at 30°C, and not at all at 25°C and at 60°C. The manner of spore formation of this organism suggested its resemblance to *Micromonospora*. Miehe also reported, however, that he saw thermophilic actinomycetes which formed spores according to the manner described by Gilbert. This suggests the probability that he had representatives of the two different groups. Schütze (1908) found, in decomposing clover hay, representatives of these two thermophilic actinomycetes, one of which appeared to belong to the *Streptomyces* group and the other to the *Micromonospora*.

Several methods for isolation and cultivation of thermophilic actinomycetes have been described by Henssen (1957). Uridil and Tetrault (1959) suggested the incorporation of colloidal silica in a highly proteinaceous medium for the growth of these organisms.

The various thermophilic forms have often been classified under the common name of "*Actinomyces thermophilus*." Waksman *et al.* (1939) demonstrated, by direct microscopic studies, that these organisms are very abundant in high temperature composts of stable manures and plant residues. Six distinct types or species were recognized, belonging to two genera, one now known as *Streptomyces* and the other as *Micromonospora*. Two of the first group (*S. thermophilus* and *S. thermofuscus*) and three of the second (*M. vulgaris*, *M. chalcea*, and *M. fusca*) were isolated and cultivated. Henssen (1957) confirmed these observations and created several new genera and species, to include these and other forms (Fig. 59).

Kosmatchev (1959) emphasized the need for separating the thermophilic from the mesophilic actinomycetes, since the former grow at 55°C and the latter cannot be adapted to grow at that temperature or under thermophilic conditions. The thermophilic actinomycetes form a sharply defined ecological and widely distributed group of microorganisms, and retain their thermophilic properties under laboratory conditions. They were believed to comprise independent species. He suggested, however, that no specific thermophilic genera be recognized, but that they should be included among the mesophilic forms.

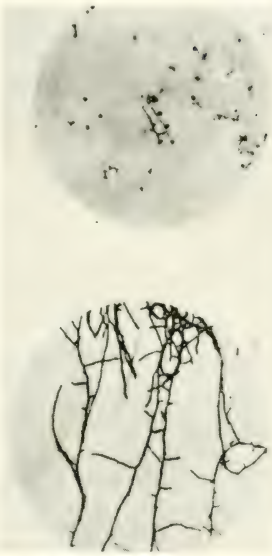

FIGURE 58. First photograph of a *Thermoactinomyces* (Reproduced from: Tsiklinsky, P. Ann. inst. Pasteur 13: 500–505, 1899).

Henssen (1957) proposed a distinct system for classifying thermophilic actinomycetes. This system is used, with certain modifications, in this treatise.

Classification of Thermophilic Actinomycetes

A. Substrate mycelium unseptated. Spores produced on aerial mycelium only.

1. Aerial hyphae branched; they are almost completely transformed into spore chains *Streptomyces* Waksman and Henrici

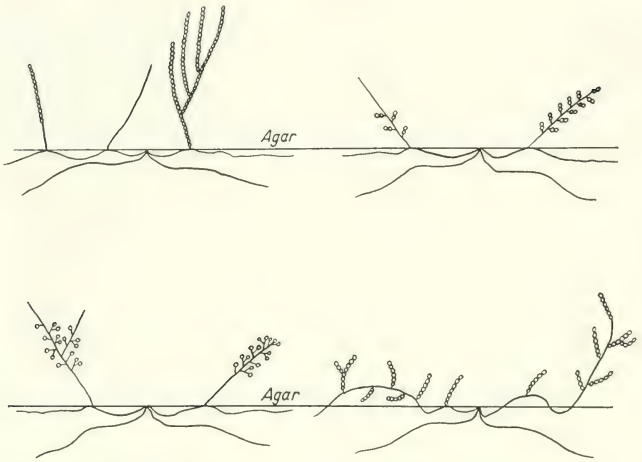

FIGURE 59. Four types of thermophilic actinomycetes (Reproduced from: Henssen, A. Arch. Mikrobiol. **26:** 379, 1957).

II. Long sterile aerial hyphae forming single spores or chains of spores on side branches.
1. Spores produced singly on simple or branched sporophores.
Thermomonospora Henssen
2. Spores produced in two's or in longer chains.
Thermopolyspora Henssen
III. Single spores or chains of spores originate directly from substrate mycelium, which sometimes emerges from the agar surface like an arch.
Thermoactinomyces Tsiklinsky
B. Substrate mycelium septated. Spores formed from both aerial and substrate mycelium.
Pseudonocardia Henssen

Genus *Streptomyces*

XVI. *Series* Thermophilus

Spores produced in chains, comparable to the mesophilic species of *Streptomyces*.

I. Sporophores and chains of spores straight.
a. Aerial mycelium white to light gray.
2. *Streptomyces rectus*
b. Aerial mycelium white; thermotolerant.
1. *Streptomyces casei*
II. Sporophores and chains of spores not straight.
1. Spore chains spiral-shaped.
6. *Streptomyces thermovulgaris*
2. Spore chains bent or curved.
a. Aerial mycelium white to violet-gray.
5. *Streptomyces thermoviolaceus*
b. Aerial mycelium gray to lavender.
4. *Streptomyces thermofuscus*
c. Aerial mycelium white to light gray.
3. *Streptomyces thermodiastaticus*

1. *Streptomyces casei* (Bernstein and Morton, 1934) nov. comb. (Bernstein, A. and Morton, H. E. J. Bacteriol. **27:** 625, 1934).

Morphology: Sporophores straight, 0.5 to 0.7 μ in diameter.

Agar media: Growth colorless to white. Aerial mycelium white.

Gelatin: Liquefaction rapid.

Milk: Positive coagulation and peptonization.

Starch: No hydrolysis.

Nitrate reduction: None.

Temperature: Optimum 40–60°C. Highly resistant to higher temperatures and to disinfectants. Thermal death point 100°C.

Habitat: Pasteurized cheese.

Remarks: Krassilnikov (1949) placed this

culture in the same group with *A. invulnerabilis* (Acosta and Rossi) Berestnew, 1897, the latter said to be even more resistant to high temperatures and to disinfectants.

2. *Streptomyces rectus* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Not *A. rectus* Krassilnikov.

Synonym: *Streptomyces thermophilus.*

Morphology: Sporophores straight, 36 to 60 μ long. Spores round or oval, 0.9 to 1.2 μ.

Sucrose nitrate agar: Growth moderate. No aerial mycelium.

Glycerol-asparagine agar: Growth good. Aerial mycelium moderate, white to light gray.

Meat–extract agar: Growth good. Aerial mycelium light gray. Soluble pigment brown.

Cellulose-dextrin agar: Growth more or less heavy. Aerial mycelium white-gray.

Potato: Growth colorless. Soluble pigment brown.

Starch: Slow hydrolysis.

Nitrate reduction: Positive.

Milk: Coagulated; not peptonized in 9 days.

Gelatin: Not liquefied (Waksman *et al.* (1939) obtained liquefaction).

Habitat: Fresh horse manure.

Remarks: Thermotolerant mesophile. Grows equally well under aerobic and anaerobic conditions.

The name "thermophilus" for this species was not recognized by Henssen (1957), since it was first used by Berestnew (1897) for another actinomycete, apparently also a species of *Streptomyces,* which was distinguished from *S. rectus* by spiral-forming chains of aerial spores, gray or dark green aerial mycelium, and yellow to dark brown colonies. Noack (1912) described an organism, under the name "thermophilus," which produced a soluble red pigment. Miehe (1907) and Schütze (1908) also described organisms under this name.

3. *Streptomyces thermodiastaticus* (Bergey, 1919) nov. comb. (Bergey, D. H. J. Bacteriol. **4:** 301, 1919).

Morphology: Sporophores form spirals. Spores spherical to oval, 0.9 by 0.7 or 0.8 μ.

Synthetic agar: Growth colorless. Aerial mycelium well developed, white.

Potato: Growth brownish. Aerial mycelium light gray.

Gelatin: Liquefaction slow.

Milk: No coagulation; no peptonization.

Starch: Strong hydrolysis.

Cellulose: Growth good.

Nitrate reduction: Positive.

Sucrose: Inverted.

Temperature: Optimum, 65°C.

Habitat: Mouth of rabbit. Soil.

4. *Streptomyces thermofuscus* (Waksman *et al.,* 1939) nov. comb. (Waksman, S. A., Umbreit, W. W., and Cordon, T. C. Soil Sci. **47:** 49, 1939).

Morphology: Aerial mycelium gives rise to spiral-shaped sporophores; spores spherical.

Sucrose nitrate agar: At 28°C, growth poor, deep gray, with but little aerial mycelium. At 50°C, growth black to violet, with gray to lavender aerial mycelium. Soluble pigment brown.

Potato: Growth abundant, brown-colored. Aerial mycelium, none or a few white patches. Soluble pigment black.

Gelatin: Liquefied. At 50°C, a grayish ring is produced and a soluble pigment is formed. At 28°C, there is growth without any soluble pigment.

Milk: Peptonized.

Starch: Hydrolysis.

Temperature: Good growth at 50 and 60°C. Will grow at 65°C. Faint growth at 28°C.

Habitat: Horse manure.

Remarks: This species is characterized by brown-colored aerial mycelium on synthetic media, spiral-shaped sporophores, and ability to grow readily at 65°C.

5. *Streptomyces thermoviolaceus* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Substrate mycelium slightly branched. Sporophores curved, 20 to 40 μ long. Spores oval, 1.0 to 1.2 by 1.2 to 1.6 μ. Facultative aerobe.

Synthetic agar: Growth slight. Sporophores ochre-brown. Aerial mycelium white to violet-gray.

Glycerol-asparagine agar: Growth yellow. Aerial mycelium abundant. Soluble pigment violet.

Nutrient agar: Growth good. No aerial mycelium.

Cellulose-dextrin agar: Growth slight. Aerial mycelium produced.

Potato: Growth good, black-violet. Aerial mycelium white. Soluble pigment dark violet.

Starch: Rapid hydrolysis.

Nitrate reduction: Negative.

Milk: Coagulation rapid; peptonization rapid.

Gelatin: Growth orange-yellow. Liquefaction positive. Soluble pigment produced.

Habitat: Fresh horse or swine manure.

Remarks: This species was divided by Henssen into two subspecies: (a) *pingens*, producing a violet pigment on potato; (b) *apingens*, producing no pigment on potato.

6. *Streptomyces thermovulgaris* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Mycelium shows little branching. Sporophores produce spirals, 20 to 40 μ long. Spores oval, 1.0 to 1.2 by 1.1 to 1.5 μ. Facultative aerobe.

Sucrose nitrate agar: Growth slight. Aerial mycelium present or absent.

Glycerol-asparagine agar: Growth moderate to good. Aerial mycelium white to black-violet.

Nutrient agar: Growth good, colorless. Aerial mycelium violet-gray. No soluble pigment.

Cellulose-dextrin agar: Growth good. Aerial mycelium produced.

Potato: Growth good in places. Aerial mycelium white to gray in spots. Soluble pigment often black.

Starch: Rapid hydrolysis.

Nitrate reduction: Positive.

Milk: Rapidly coagulated and peptonized.

Gelatin: Liquefaction varies.

Temperature: Grows well at 40–50°C, somewhat better under anaerobic than aerobic conditions. At 28°C growth slight; at 60°C growth moderate. No aerial mycelium at 28°C; slight aerial mycelium at 60°C.

Habitat: Fresh compost and fresh horse manure.

Genus *Thermomonospora*

The type species is *Thermomonospora fusca* (Waksman *et al.*) Henssen.

These organisms produce colorless or yellow growth on agar media. Substrate mycelium nonseptated. The aerial mycelium is sharply limited and white. Aerial hyphae are simple or branched, developing as side or terminal branches on the substrate hyphae. The spores are formed singly on simple or branched sporophores on the unbranched aerial hyphae. Spore formation is acropetal. Gram-positive. Not acid-fast. Thermophilic, facultative aerobes.

I. Spore-masses produced in form of a head or a bunch of grapes.
    2. *Thermomonospora fusca*
II. Spore-masses spiked or entangled.
    3. *Thermomonospora lineata*
III. Spores mostly single on simple or branched sporophores.
    1. *Thermomonospora curvata*

1. *Thermomonospora curvata* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Aerial hyphae 30 to 50 μ long. Mass of spores clumped or hairy. Spores round, 1.2 to 1.8 μ.

Agar media: Colonies colorless or yellow.

Aerial mycelium chalk-white. Cultures are thermophilic, facultatively aerobic. Aerial spores produced in 3 to 4 days in hanging drops. Spores formed on simple or branched sporophores. Spores oval, later round.

Sucrose nitrate agar: Growth colorless. Aerial mycelium moderate.

Glycerol-asparagine agar: Growth abundant, yellow. Aerial mycelium produced.

Nutrient agar: Growth good, yellow. Aerial mycelium thick.

Potato: Growth yellow, partly covered with aerial mycelium. Soluble pigment light brown.

Starch: No hydrolysis.

Nitrate reduction: Weak.

Milk: Unchanged in 16 days.

Gelatin: Not liquefied.

Temperature: Optimum growth at 50°C. Limited growth at 28 and 65°C.

Habitat: Fresh cow manure and manure compost.

2. *Thermomonospora fusca* (Waksman *et al.*, 1939) Henssen (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Aerial hyphae 20 to 30 μ long. Spores round 1.5 to 2.0 μ. Since the spores are not produced on the substrate mycelium as in *Micromonospora*, but exclusively on the aerial mycelium, the species was transferred from *Micromonospora fusca* to *Thermomonospora fusca*. The branching of the substrate mycelium is monopodial. The hyphae are long, straight, and form straight side branches. The branching is so characteristic, as compared to all the other thermophilic species, that this species can easily be recognized. The aerial mycelium is colored brown (Fig. 60).

Sucrose nitrate agar: Growth at 28°C deep gray; at 50°C, growth is dark brown to violet. Aerial mycelium gray to lavender. Soluble pigment brown.

Gelatin: Liquefied.

Potato: Growth brown-colored. No aerial mycelium. Soluble pigment black.

FIGURE 60. *Thermomonospora fusca* (Reproduced from: Henssen, A. Arch. Mikrobiol. **26:** 401, 1957).

Milk: No coagulation; slight peptonization.

Habitat: Horse manure.

Starch: Hydrolysis.

Nitrate reduction: Slight.

Cellulose: Growth good.

Temperature: Growth and aerial mycelium formation are good at 50–65°C.

3. *Thermomonospora lineata* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Sporophores straight, 50 to 80 μ long; spores round, 1.5 to 2.0 μ. Spore chains hairy or clumpy (Fig. 61).

Culture was isolated on nutrient agar but was not obtained in pure state. Aerial hyphae mostly branched. Spores produced on simple or branched sporophores. Substrate hyphae monopodial and branched. Hyphae are not as long as in the case of *T. fusca*. Side branches are more compact.

Optimum growth: 50–60°C.

Habitat: Composted sheep manure.

Genus *Thermopolyspora*

Growth on agar media colorless to yellow. Substrate mycelium not septated. Aerial mycelium limited, white. Aerial hyphae not branched, developing in the form of side or terminal branches of the substrate hyphae. Spores produced in short chains on unbranched sporophores. Spore formation acropetal. Spore chains unbranched, straight,

FIGURE 61. *Thermomonospora lineata* (Reproduced from: Henssen, A. Arch. Mikrobiol, **26:** 401, 1957).

bent or spiral-shaped. Gram-positive, non-acid-fast, thermophilic, facultatively aerobic.

This genus comprises two species: *T. bispora* with chains made up of double spores, and *T. polyspora* with more than two spores.

Usually only the side branches of the substrate mycelium grow into aerial hyphae. The chains of spores are produced from the unbranched sporophores around the aerial hyphae.

Type species: *Thermopolyspora bispora* Henssen.

1. *Thermopolyspora bispora* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Aerial hyphae 20 to 30 μ long, with 20 to 40 spore chains. Spores are double, round, 0.9 to 1.3 μ.

Cultural characters: Colonies mostly colorless. Aerial mycelium chalk-white. Organism thermophilic, facultatively aerobic.

The following properties are discussed on the basis of anaerobic growth at 60°C.

Sucrose nitrate agar: Sparse growth.

Glycerol-asparagine agar: Growth slight or abundant. No aerial mycelium.

Nutrient agar: Good growth. Aerial mycelium thick.

Starch agar: No hydrolysis.

Gelatin: Unchanged.

Potato: Individual colonies without aerial mycelium.

Nitrate reduction: Negative.

Milk: Not changed after 7 days.

Habitat: Fresh cow and swine manure or composted sheep manure.

Type culture: IMRU 3759.

2. *Thermopolyspora polyspora* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Aerial hyphae 45 to 50 μ long, 1.0 to 1.3 μ thick. Sporophores straight to spiral-forming, individual or in groups. Spores round, 3 to 10 by 1.1 to 1.8 μ.

Physiological properties of spore-free cultures, at 60°C under aerobic conditions: Colonies yellow, aerial mycelium dirty white.

Glycerol-asparagine agar: Growth orange-yellow.

Cellulose-dextrin agar: Growth colorless.

Nutrient agar: Growth yellow.

Potato: Growth yellow.

Remarks: These cultures resemble, through their colored compact colonies, the spore-free cultures of *Micromonospora*. Spiral formation has resulted in the confusion of *T. polyspora* with species of *Streptomyces*.

Genus *Thermoactinomyces*

The genus *Thermoactinomyces* is similar in some respects to the genus *Micromonospora*;

it is distinct from it, however, in the formation of a typical aerial mycelium.

Henssen emended this genus as follows: Colonies on agar colorless or yellow to orange. Substrate mycelium not septated. Aerial mycelium not sharply delimited; white or bluish-green. Aerial hyphae are simple or branched, formed as terminal or side branches; they may also be curved; they grow upwards from the substrate mycelium. Spores, single or in chains, remain on the unbranched aerial hyphae. The organisms are thermophilic, capable of growing at 50–65°C. They are aerobic or facultatively aerobic. Some have their optimum at 60°C.

Type species: *Thermoactinomyces thalpophilus* Waksman and Corke.

The genus can be classified as follows:

I. Aerial mycelium white.
  a. No soluble pigment.
    6. *Thermoactinomyces vulgaris*
  b. Soluble wine-colored to rose pigment in certain media.
    3. *Thermoactinomyces thalpophilus*
II. Aerial mycelium grayish-green.
  2. *Thermoactinomyces monosporus*
III. Aerial mycelium scant, white to bluish-green.
  a. No soluble pigment.
    1. *Thermoactinomyces glaucus*
  b. Soluble pigment green.
    5. *Thermoactinomyces viridis*
IV. Aerial mycelium white to dark gray.
  4. *Thermoactinomyces thermophilus*

1. *Thermoactinomyces glaucus* Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Spore chains simple or branched, about 7 μ long, containing 4 to 10 spores. The chains are straight or bent. Spores round or oval, 0.9 to 1.4 by 0.5 to 1.9 μ.

Cultural properties: Growth almost colorless; aerial mycelium white to bluish-green. Facultative aerobe.

Sucrose nitrate agar: Growth moderate. Aerial mycelium moderate.

Glycerol-asparagine agar: Growth moderate. Aerial mycelium scant.

Nutrient agar: Growth sparse.

Cellulose-dextrin agar: Growth good. Aerial mycelium abundant, white to green. Cellulose decomposed very actively.

Gelatin: Unchanged.

Potato: Individual colonies without aerial mycelium.

Starch: Slow hydrolysis.

Nitrate reduction: Positive.

Milk: Slowly coagulated and peptonized.

Habitat: Composted sheep manure.

2. *Thermoactinomyces monosporus* (Lehmann and Schütze) Waksman (Schütze, H. Arch. Hyg. **67:** 35, 1908; after Krassilnikov, 1941).

Morphology: Substrate hyphae about 1.0 μ in diameter. Oval spores 1.5 to 1.8 by 1.0 to 1.4 μ produced singly.

Agar media: Growth yellowish, compact, smooth or lichenoid. Aerial mycelium grayish-green. Good sporulation of hay infusion-peptone agar; somewhat less on glycerol-peptone and lactose-peptone agar; none on peptone-glucose agar.

Potato: No growth.

Gelatin: Liquefaction positive.

Milk: No coagulation or peptonization.

Blood serum: Good, smooth growth; liquefaction positive.

Temperature: Optimum 37–55°C; grows poorly at 27°C and not at all at 60°C.

Habitat: Self-heated hay.

Remarks: Henssen (1957) considers this species as more closely related to the genus *Thermomonospora.*

3. *Thermoactinomyces thalpophilus* Waksman and Corke, 1953 (Waksman, S. A. and Corke, C. J. Bacteriol. **66:** 377, 1953).

Morphology: Spores, produced singly or in short chains, are round, 0.8 to 1.2 μ.

Agar media: Grows equally well on a variety of media under aerobic and anaerobic conditions. Colonies colorless to orange.

Aerial mycelium white. Soluble wine-red pigment produced on sugar-containing salt media.

Sucrose nitrate agar: Very little growth.

Glycerol-asparagine agar: Growth abundant. Aerial mycelium formed, orange.

Nutrient agar: Growth yellow. Aerial mycelium limited.

Gelatin: Some liquefaction.

Potato: Growth limited. Aerial mycelium limited.

Starch: Rapidly hydrolyzed.

Nitrate reduction: Positive.

Milk: Coagulation rapid; peptonization rapid.

Temperature: Grows well at 50–60°C, lesser growth at 40°C; no growth at 28 and 65°C.

Habitat: Manure compost.

4. *Thermoactinomyces thermophilus* (Berestnew, 1897) nov. comb.

Morphology: Sporophores straight (Noack, Waksman *et al.*) or spiral-shaped (Krassilnikov). Spores spherical.

Cultural properties: Substrate growth yellow-brown. Soluble pigment brown. Aerial mycelium white to dark gray.

Synthetic agar: Growth colorless. Aerial mycelium thin white. No soluble pigment.

Potato: Growth yellowish. No aerial mycelium. Soluble pigment brown.

Gelatin: Liquefaction rapid. No pigmentation.

Milk: Coagulation and peptonization.

Starch agar: Growth yellowish. Aerial mycelium powdery, white-gray. Starch rapidly hydrolyzed.

Nitrate reduction: Rapid.

Cellulose: Slight growth.

Temperature: 35–55°C. Optimum 50°C.

Remarks: Noack described, under this name, organisms of a bright red color, with a red soluble pigment. Optimum 40–59°C. Miehe and Schütze also described organisms under this name.

5. *Thermoactinomyces viridis* Schuurmans *et al.*, 1956 (Schuurmans, D. M., Olson, B. H., and San Clemente, C. L. Appl. Microbiol. **4:** 61–66, 1956).

Morphology: Spores borne singly; oval (about 1.0 by 1.3 μ). Diameter of hyphae approximately 0.5 μ.

Glucose-asparagine agar. No growth.

Calcium malate agar: Colonies colorless, 1 to 2 mm in diameter; a few colonies with blue-green aerial mycelium.

Nutrient agar: Growth wrinkled, colorless, and close to agar surface. Aerial mycelium blue-green. Soluble pigment emerald-green.

Glucose-peptone agar: Colonies colorless, 1 to 2 mm in diameter. No aerial mycelium.

Nutrient broth: Flocculent cream-colored submerged growth; surface growth blue-green. Soluble pigment green.

Gelatin: Liquefied.

Potato: No growth.

Milk: Coagulation positive, followed by peptonization.

Nitrate reduction: Negative.

Starch: Hydrolysis.

Temperature: Minimum, 37°C; optimum, 55°C; maximum, 60°C.

Antagonistic properties: Produces the antibiotic thermoviridin, active primarily against gram-positive bacteria.

Habitat: Composted manure pile.

Remarks: Description of growth and biochemical reactions after 14 days' incubation

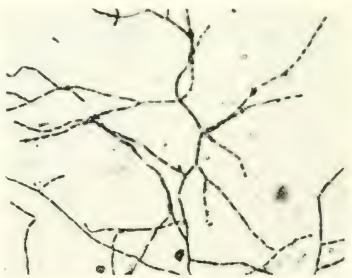

FIGURE 62. *Pseudonocardia thermophila* (Reproduced from: Henssen, A. Arch. Mikrobiol. **26:** 409, 1957).

at 45°C. The organism in many ways resembles *Thermoactinomyces monosporus*. The points of difference, however, are considered significant, the latter possessing hyphae with a diameter of about 1 μ, an optimum growth range of 37 to 55°C, and failing to coagulate milk.

6. *Thermoactinomyces vulgaris* Tsiklinsky, 1899 (Tsiklinsky, P. Ann. inst. Pasteur **13:** 500, 1899).

Synonym: *Micromonospora vulgaris* Waksman *et al.*, 1939; Erikson, 1953.

Morphology: Substrate mycelium fine, 0.5 μ in diameter. Spherical and oval spores are borne singly at the ends of short branches, from which they are easily broken. They often appear to sit directly on mycelium.

Sucrose nitrate agar: Growth colorless. Aerial mycelium white.

Nutrient agar: Growth good. Aerial mycelium white.

Potato: Growth good.

Gelatin: Liquefaction positive.

Milk: Coagulation and peptonization.

Starch: Hydrolysis positive.

Cellulose: No decomposition.

Nitrate reduction: Negative.

Sucrose: Not inverted.

Temperature: Grows at 48–68°C; optimum at 57°C.

Source: Human and animal excreta, high temperature composts, self-heated hay, soil.

Remarks: Resembles mesophilic members of the genus *Micromonospora*, except that it produces an aerial mycelium which forms single spherical spores. The aerial phase of the development of this organism is believed to be intimately associated with its thermophilic nature (Erikson, 1952, 1953, 1955a). Oxygen concentration has an important effect upon the growth of the aerial mycelium of this organism (Webley, 1954). The effect of composition of medium and the growth-temperature relationships of this organism were studied recently by Tendler (1959).

Genus *Pseudonocardia*

Substrate mycelium septated. Spores produced in substrate and in aerial mycelium.

On the basis of its morphology, *Pseudonocardia* should be placed between *Streptomyces* and *Norcardia*. Along with *Nocardia*, it has the common property of septation of the substrate mycelium, but no fragmentation. In common with *Streptomyces*, it produces aerial mycelium which is thicker than the substrate mycelium, and which changes into long spore chains. It differs from *Streptomyces* in being unable to hydrolyze gelatin or starch.

Type species: *Pseudonocardia thermophila* Henssen.

Pseudonocardia thermophila Henssen, 1957 (Henssen, A. Arch. Mikrobiol. **26:** 373–414, 1957).

Morphology: Substrate mycelium septated. Spore formation in substrate mycelium. Aerial hyphae unbranched, formed as side branches of the substrate hyphae. Spores basipetal. Substrate spores 2.5 by 1.5 to 1.8 μ. Aerial spores produced in chains at the tip of the hyphae are 2.5 μ long; spores produced at the base of the chain are 5 μ long by 1.5 to 1.8 μ wide. Thermophilic, facultative aerobe (Fig. 62).

Sucrose nitrate agar: Growth yellow. Aerial mycelium limited, white.

Glycerol-asparagine agar: Growth moderate, yellow. Aerial mycelium produced, white.

Nutrient agar: Growth good, yellow. Aerial mycelium in thick colonies.

Potato: Colonies individual, yellow. No aerial mycelium. Soluble pigment yellow.

Gelatin: Not liquefied.

Starch: No hydrolysis.

Nitrate reduction: Positive.

Milk: Unchanged in 16 days.

Habitat: Fresh horse manure.

Chapter 12

Actinoplanaceae

Substrate mycelium usually inconspicuous, formed in water, on a variety of plant and animal materials. Aerial mycelium usually lacking; only certain species produce such mycelium, thus resembling *Streptomyces*. Reproduction by spores formed in sporangia. The spores in *Actinoplanes* possess flagella and are motile. The spores of *Streptosporangium* are without flagella and are nonmotile. Many species produce aerial spores. These organisms can be cultivated on a variety of artificial media; they will then resemble in their growth other actinomycetes. The family is widely distributed in soil and in fresh water. The Actinoplanaceae can be classified as follows:

I. Aerial mycelium usually not formed; coiled conidiophores lacking; sporangiospores motile.

Genus I. *Actinoplanes*

II. Aerial mycelium abundant; coiled conidiophores as well as sporangia are formed in some species; sporangiospores nonmotile.

Genus II. *Streptosporangium*

Genus *Actinoplanes* Couch

(Couch, J. N. J. Elisha Mitchell Sci. Soc. **66,** 87, 1950; **71,** 48, 1955. Trans. N. Y. Acad. Sci. **16,** 315, 1954).

Occur on sterilized leaves in water, forming a very inconspicuous mycelium which branches throughout the leaf tissue. The external hyphae are scattered or in tufts on the leaf surface and form a fringe around the edge of the leaf. Aerial mycelium is lacking or sparingly formed; usually pinkish to reddish, sometimes hyaline; frequently decolorizes the green leaf and gives it a pinkish or reddish color. Hyphae slightly to considerably branched, irregularly coiled, twisted or straight, sparingly septate, 0.2 to 2.6 μ in diameter. Sporangia usually abundant on leaves, formed only when the leaf is at or close to the surface of the water, *i.e.* formed typically only in air, and appearing black under the low power of the microscope, owing to refraction; of varied sizes and shapes. Spores in coils, nearly straight chains, or irregularly arranged, in sporangia; 1 to 1.5 μ in diameter, globose or subglobose, usually slightly angular, with one to several shiny bodies, with several polar flagella, and motile; germination by a minute germ tube which branches to form a mycelium. Sporangial wall evanescent or persistent (Fig. 63).

The organisms form on various nutrient agars a brilliantly colored, tough to pasty growth. Surface very variable: smooth and even with the agar or elevated bumpy, convoluted, ridged, folded, cracked, etc., usually moist and shiny, rarely pulverulent. Hyphae of two more or less distinct forms, the submerged and the surface hyphae, the latter usually more or less vertical and in some species forming a compact "palisade." Sporangia abundant on some agars, usually formed at the surface. Spores formed in some species. On certain agars, the mycelium of some species breaks up, when crushed,

into irregular pieces of hyphae, rods and coccoid bodies.

Aerobic, gram-positive, and acid-fast.

Occur saprophytically in soils and in fresh water, and are world-wide in distribution. Over 120 cultures were isolated.

The genus *Actinoplanes* is readily distinguished from *Streptosporangium*. On leaves, the latter produces a conspicuous aerial mycelium which resembles that in most species of *Streptomyces*, whereas no such mycelium is usually found in *Actinoplanes*. The isolates of the latter grow much more vigorously on agar than do those of *Streptosporangium*. The most striking difference is that in *Actinoplanes* the sporangiospores are motile, whereas in *Streptosporangium* they are nonmotile.

Under certain conditions of culture, some species of *Actinoplanes* resemble *Micromonospora*. A nonsporangial strain of *Actinoplanes* might easily be confused with certain micromonosporas. The spores of *Micromonospora*, however, are formed singly or in grape-like clusters but never in chains, whereas in *Actinoplanes* they are formed singly and also in chains but not in grape-like clusters. In most species of *Micromonospora*, on certain agars, the sporulating surface turns black, whereas this change does not occur in *Actinoplanes*. In general, the species of *Micromonospora* are less vigorous in growth than those of *Actinoplanes*.

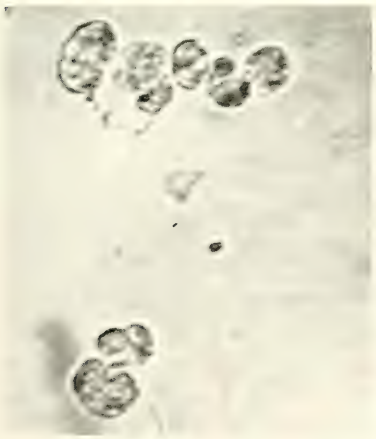

FIGURE 63. *Actinoplanes* (Prepared by H. Lechevalier, Institute of Microbiology).

Several species of *Actinoplanes*, when grown on potato-glucose and certain other agars, will form a small pasty culture which, when mounted and crushed under a coverslip, breaks up into minute spheres, irregular rods, and short, branched, hyphal segments, much as in *Nocardia*. Such growth, however, is not the normal condition for any species of *Actinoplanes*. None of the 25 species of *Nocardia* examined by Couch formed sporangia when grown either on any of the agars most favorable for sporangial formation or on *Paspalum* leaves in water.

Gaertner (1955) isolated cultures of *Actinoplanes* from soil and found them capable of decomposing keratin.

The type species is *Actinoplanes philippinensis* Couch.

Actinoplanes philippinensis Couch, 1950 (Couch, J. N. J. Elisha Mitchell Sci. Soc. **66,** 87, 1950).

Morphology: Produces a very delicate, hyaline to pinkish-buff internal mycelium and an inconspicuous external fringe of threads around the entire edge of the leaf of sterile *Paspalum* grass in water. Sometimes a compact mound or tufts of hyphae are scattered over the top surface, giving the leaf a speckled or finely powdered appearance. Hyphae are 0.5 to 1.5 μ thick, branched, sparingly septate. Sporangia, usually formed abundantly on grass after about 10 days, on long unbranched stalks, mostly spherical when mature, 8.4 to 22 μ. Spores arranged, at maturity, in coils or irregularly in the sporangium, about 1 to 1.2 μ. They are discharged through a pore or by the partial dissolution of the sporangial wall, and swim vigorously.

Sucrose nitrate agar: Growth at room temperature poor to fair, flat or slightly elevated. Margin smooth or scalloped, sectoring frequent. Color pale buff to tawny, changing in some old cultures to brown with a lighter margin. Forms a compact surface layer, made up mostly of distinct palisades, and a submerged region of loosely arranged hyphae. Surface region frequently stratose in old cultures, with narrow, orange-colored layers. Sporangia fairly abundant in some cultures, not formed in others; spherical to irregular; frequently beneath the surface in old cultures, owing to overgrowth by palisade hyphae. Sometimes a new layer of sporangia forms over the first layer. Odor slightly fragrant. Usually colors the agar pale yellow.

Glucose-asparagine agar: Growth good to very good, consisting of a central area of elevated, fine convolutions, radial ridges or bumps, and a smooth area with radial grooves gradually sloping into the submerged margin. Surface moist-appearing and glossy. Color of center apricot-orange to brown, surrounded by an ochraceous-salmon or

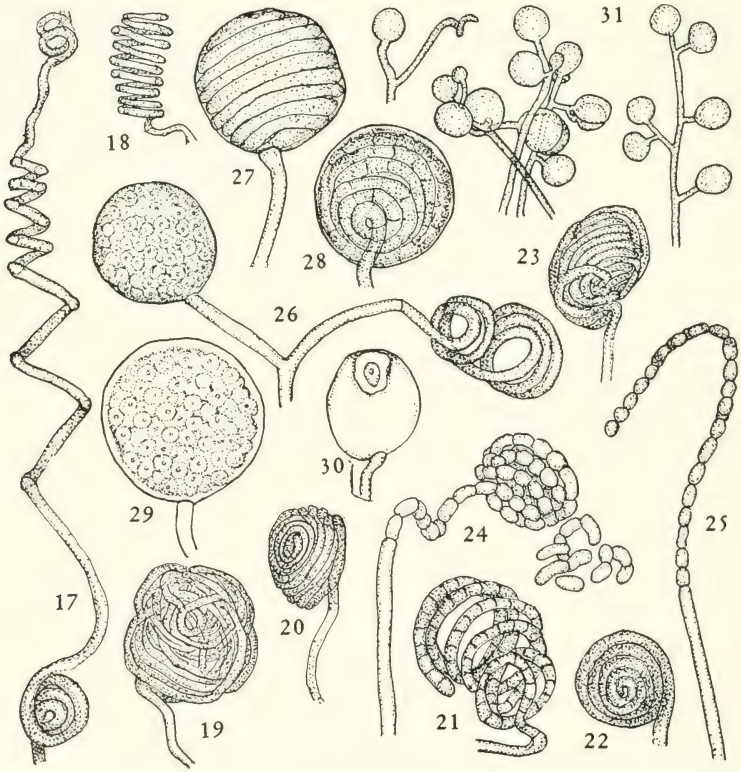

FIGURE 64. *Streptosporangium roseum* (Reproduced from: Couch, J. N. J. Elisha Mitchell Sci. Soc. 71: 152, 1955).

light ochraceous-salmon margin. Sporangia usually on the smooth areas, none on the elevated parts; formed on palisade hyphae.

Potato-glucose agar: Growth good to very good. Central area with coarse convolutions or large bumps and irregular ridges separated by radial grooves which slope to the smooth distinct margin. Surface glossy. Color apricot-orange to russet, becoming gray in old cultures. Soluble pigment darkens the agar. Sporangia formed on the margin of some cultures, absent in most. Palisades formed.

Nutrient agar: Growth fair. Center slightly elevated and with a wide flat margin. Color ochraceous-orange to cinnamon-rufous. Sporangia very rarely formed. Palisade hyphae usually not distinct.

Gelatin: Liquefied.

Habitat: Soil from Philippine Islands; also found in African soils and in marshland soils in Germany.

Remarks: This species is characterized by the predominantly spherical sporangia usually on long unbranched stalks, the rather poor and usually flat growth on synthetic agar, and the very distinct palisade hyphae on this medium. The dark brown diffusible pigment on potato-glucose agar is also characteristic.

Genus *Streptosporangium* Couch

Occurs on sterilized leaves of *Paspalum* grass in water, forming an inconspicuous mycelium which overgrows the leaves, and an aerial mycelium which grows in scattered or concentrically arranged tufts. The aerial mycelium is white to pinkish on the leaves; hyphae are much branched, sparingly septate, and about 0.5 to 1.2 μ in diameter. On some media, sporangia are formed abundantly in the aerial mycelium. Spores are abundant in the sporangia, without flagella, and are nonmotile. Growth poor to good on a variety of semisolid media (Fig. 64).

Four cultures, representing three distinct species, were found to comprise this genus. Two of the species were isolated from soil by the soil dilution method and the third from dog manure.

The type species is *Streptosporangium roseum* Couch.

Streptosporangium roseum Couch, 1955 (Couch, J. N. J. Elisha Mitchell Sci. Soc. **71**, 148, 1955).

Morphology: Grows on sterile leaves, either in soil water or on damp sterile soil, forming a substrate mycelium which spreads over the surface of the leaf, not penetrating or decolorizing it; it also spreads over the soil. Aerial mycelium white at first, changing to pale pink; it appears as single hyphae or as minute tufts which grow to form mounds up to 2 mm across, arranged more or less in concentric circles. Sporangia first appear on scattered single hyphae, apical on the main thread or on short, lateral branches, a few to many sporangia on one hypha. The sporangia are white in small groups, pink in large masses; spherical, 7 to 19 μ in diameter. Shortly after their formation, spores are visible as a single coil in each sporangium; when completely formed, they are irregularly arranged. Immersion of the mature

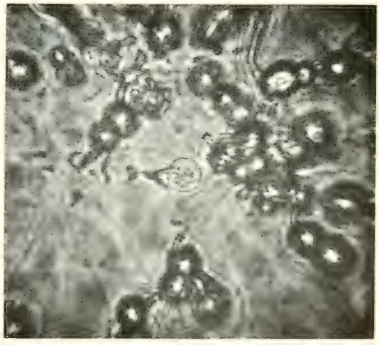

FIGURE 65. *Streptosporangium* isolated from forest litter (Reproduced from: Van Brummelen, J. and Went, J. F. Labor. Microb. Univ. Amsterdam **23**: 391, 1957).

sporangium in water brings about the swelling of an intersporal substance, causing the wall and the spores to push out on one side, forming a cone-shaped projection about half as long as the diameter of the sporangium. The spores are forcibly ejected through an opening in the cone. They are nonmotile, spherical, 1.8 to 2.0 μ in diameter, with one shiny globule. Sporangial wall is persistent for several hours after spore discharge. In addition to sporangia, spores are also formed in coils somewhat as in *Streptomyces*, though the coils are much less conspicuous (Fig. 65).

Sucrose nitrate agar: Colony usually flat, level with agar surface; concentric zonation distinct or absent. Surface glossy or powdery. Color usually white, sometimes pinkish-buff or cream-buff. Sporangia absent to fairly abundant, always formed some distance above the surface of the agar. In some cultures, coils form which break up into spores as in *Streptomyces*.

Glucose-asparagine agar: Growth poor, slightly elevated and minutely ridged, sloping to the fimbriate margin. Surface of central region powdery with white aerial hyphae. Sporangia absent.

Potato-glucose agar: Growth usually good, center elevated with irregular bumps and ridges; margin flat and even with surface of agar. Color of colony at first creamy, becoming tawny and then brown, after which white floccose spots of hyphae appear, usually spreading to cover the entire culture. Sporangia usually formed in vast numbers, the white areas becoming rosy pink as the sporangia mature; the pinkish areas are frequently minutely pocked. Surface moist at first, appearing dry and floccose as aerial hyphae and sporangia are formed. Agar colored reddish-brown with a vinaceous tinge.

Nutrient agar: Growth fair, color usually cream-buff, rarely buff-brown. Surface usually glossy, sometimes powdery with aerial hyphae which may be united to form many upright fascicles. Sporangia absent.

Habitat: Garden soil in North Carolina and forest litter in Holland and Denmark (Van Brummelen and Went, 1957).

Remarks: Nonomura and Ohara (1960b) found the genus *Streptosporangium* widely distributed in the soils of Japan. In addition to the original *S. roseum* Couch, four new species were isolated: *S. album*, *S. viridialbum*, *S. amethystogenes*, and *S. vulgare*. These were classified on the basis of the color of the aerial mycelium and formation of soluble pigment on oatmeal agar media.

Chapter 13

Incompletely Described Species of Actinomycetes

Numerous isolates of cultures of actinomycetes listed in the literature, under a variety of different generic and specific names, could not be identified at present, due to insufficient descriptions. They are reported here as "incompletely described." The naming of such cultures was based either on a casual observation or on the assumed occurrence of such an organism in a certain disease condition. Frequently the particular organism was not even obtained in pure culture, but was given a name, often for the mere purpose of obtaining for the author credit for the particular isolation or observation. In other cases, it is fairly certain that the culture said to have been isolated from a disease condition was nothing more than a dust contamination.

In the preparation of this list, the author has used freely the carefully collected records of Brumpt (1939), Dodge (1935), Baldacci (1944), and Krassilnikov (1949). Very few of these descriptions were complete enough to include the recorded cultures among the readily identifiable species. This is particularly true, even in recent years, where media of unknown composition were used for descriptive purposes.

Not all the synonyms are recorded here. For further detail, the reader is referred to the reviews of Chalmers and Christopherson (1916), and Dodge (1935),* especially for the pathogenic or largely would-be pathogenic organisms, and to Brumpt (1939) and Krassilnikov (1949) for the nonpathogenic forms. Only those synonyms that would tend to throw light upon the systematic position of the culture are listed here.

Since the name "*Actinomyces*" has been largely used for the incompletely described cultures, it is left as such, and the list is recorded in the order of species. Where the name *Nocardia* was originally used, it is either reported as a synonym of "*Actinomyces*," or under *Nocardia*. The same principle was applied to *Micromonospora* and other well-recognized genera.

No serious attempt was made to record all the other listings of cultures believed to be actinomycetes, especially those that have been insufficiently described under a variety of different names, such as *Cladothrix*, *Discomyces*, etc. Most of these names appear to be synonyms of those listed above. Only a few of the names listed under *Streptothrix* and *Oospora* are included under the "*Actinomyces*." No effort was made to list cultures for which only a generic name was given without any specific designation, or which were recorded by a number only.

A large number of species have recently been listed under the genus *Streptomyces* without any description at all or with a totally insufficient description. Frequently

*Some of these compilations may be designated, quite properly, in the words of Erikson (1940), as "veritable mausoleums wherein the errors of the past are indiscriminately embalmed."

the description is credited to a company and not to an individual scientist. This was done primarily as an effort to establish priority for an antibiotic isolated from such a culture, or for patent purposes. These designations are placed in a separate category, with emphasis on the antibiotic. A group of cultures described by Gause *et al.* (1957) has also been placed in a separate category, since no decision can be reached as yet concerning their synonymity with previously described species.

For more complete lists of names of actinomycetes, comprising both genera and species, the reader is referred to Buchanan and Lessel (1959), Lessel (1960), and especially to their forthcoming treatise "Index Bergeyana."

According to Buchanan and Lessel (1959), there are now about 3000 names given to different strains of bacteria that are recognized as belonging to one or another of the genera of the order Actinomycetales. They emphasize, however, that "this is not to be interpreted as meaning that there are three thousand species, for a large proportion of the names (probably two-thirds) are not available for use because they were not validly published, or are homonyms, or synonyms, or are not binary combinations, or were proposed as hypothetical names, or were insufficiently described and are to be regarded as naked names (*nomina nuda*) or as doubtful names (*nomina dubia*) or are officially rejected names, or because illegitimate as contravening some other nomenclatural rule."

An attempt has been made to present in the following lists some of the incompletely described forms of actinomycetes. Many additional names are found in the previous chapters as synonyms of well described organisms.

The first list comprises the forms described under the names *Actinomyces* (*A*), *Streptothrix* (*St.*), or *Oospora* (*O*).

The name *Actinomyces* used in this list is not to be confused with the genus *Actinomyces* recognized at present, although some of the cultures so designated here would no doubt be considered as members of this genus.

A. acidoresistans, a culture obtained from Pribram collection in Vienna. IMRU 3049.

A. actinoides (Smith, 1918) Bergey, 1923.

A. actinomorphus (Gray and Thornton, 1928) Bergey, 1930.

St. actinomyces Rossi-Doria, 1891.

St. actinomycotica Foulerton, 1899.

A. aerugineus Wollenweber, 1920.

A. agrestis (Gray and Thornton, 1928) Bergey, 1930.

St. albido Chester, 1901.

A. albidoflava (Rossi-Doria, 1891) Ford, 1927

A. albidofuscus Berestnew, 1897.

A. albido-fuscus Neukirch, 1902.

A. alboatrus Waksman and Curtis, 1916.

A. albopurpureus Duché, 1934.

A. albus-acidus Neukirch, 1902.

A. albus var. *acidus* Nannizzi Pollacci, 1934.

A. albus var. *alfa* Ciferri, 1927.

A. albus asporogenes Berestnew, 1897.

A. albus chlamydosporus Krassilnikov, 1949.

A. albus vulgaris Krassilnikov, 1941.

St. alpha Price-Jones, 1900.

A. allenbachi Sartory and Meyer, 1932.

A. almquisti Duché, 1934.

A. americanus (*Cohnistreptothrix americana* Chalmers and Christopherson, 1916) Dodge, 1935.

A. anaerobies (Plaut, 1920; *Oospora anaerobies* Sartory, 1923) Dodge, 1935.

A. anaerobicus (Plaut, 1920) Ford, 1927.

St. aquatilis Johan-Olsen, 1893.

A. aquatilis Salimowokaja, 1928. Krassilnikov states that it is related to *A. glaucus*.

A. arborescens (Edington, 1887) Gasperini, 1894.

A. aromaticus Krassilnikov, 1941.

A. asteroides var. *serratus* Sartory and Meyer, 1930.

A. atypica pseudoturberculosa Hamm and Keller, 1909.

St. aurea (Saint-Sévérin, 1895) Ford, 1927.

A. aureus Lachner-Sandoval, 1898.

Syn. *N. aurea* Castellani and Chalmers, 1913.

A. avadi Dodge, 1935.

A. baarnensis Duché, 1934.

Syn. of *A. viridis* Duché, 1934.

A. bahiensis (daSilva, 1919) Brumpt, 1927.

A. bellisari Dodge, 1935.

A. berestneffi (Chalmers and Christopherson, 1916) Brumpt, 1939.

Syn. *N. berestneffi* Chalmers and Christopherson, 1916.

A. berardinisi (Namyslowsky, 1912) Brumpt, 1939.

St. beta Price-Jones, 1900.

A. bicolor Trolldenier, 1903.

Syn. *N. bicolor* deMello and Fernandes, 1919.

A. bolognesii-chiurcoi (Vuillemin) Dodge, 1935.

A. bostroemi Baldacci, 1937.

A. bovis albus Gasperini, 1894.

St. bovis communis Foulerton and Price-Jones, 1901.

A. bovis farcinicus Gasperini, 1894.

A. bovis luteoroseus Gasperini, 1894.

A. bovis sulfureus Gasperini, 1894.

A. bovis var. *nigerianus* Erikson, 1935.

A. bronchialis (Sartory and Lasseur, 1914) Brumpt, 1939.

A. bronchiticus (Castellani *et al.*, 1921) Brumpt, 1939.

Syn. *Anaeromyces bronchitica* Castellani *et al.*, 1921.

A. brumpti (Bordjoski and Milochevitch, 1935) Brumpt, 1939.

A. bruni (Chalmers and Christopherson, 1916) Brumpt, 1939.

Syn. *N. bruni* Chalmers and Christopherson, 1916.

A. buccalis (Roger *et al.*, 1909) Brumpt, 1939.

Syn. *O. buccalis* Roger *et al.*, 1909.

N. buccalis Castellani and Chalmers, 1913.

A. cameli (Mason, 1919) Ford, 1927.

A. caminiti Ford, 1927.

St. candida (Petruschky, 1898) Ford, 1927.

Syn. *N. candida* Castellani and Chalmers, 1913.

A. canis (Rabe, 1888) Gasperini, 1894.

A. canis familiaris Rivolta, 1884.

St. caprae (Silberschmidt, 1899) Ford, 1927.

A. carnea (Rossi-Doria, 1891; Gasperini, 1894; Kruse, 1896) Ford, 1927.

Syn. *N. carnea* Castellani and Chalmers, 1913.

A. carougeaui (Gougerot, 1909) Brumpt, 1939.

Syn. *N. carougeaui* Castellani and Chalmers, 1913.

A. catarrhalis (Bailly, 1921) Brumpt, 1939.

A. cati (Rivolta, 1878) Gasperini, 1894.

A. caviae (Snijders, 1924) Erikson, 1935.

A. cerebriformis (Namyslowsky, 1910) Brumpt, 1939.

A. cereus Lieske, 1921.

A. chalmersi (deMello and Fernandes, 1919) Dodge, 1935.

Syn. *N. chalmersi* deMello and Fernandes, 1919.

A. christophersoni (deMello and Fernandes, 1919) Dodge, 1935.

Syn. *N. christophersoni* deMello and Fernandes, 1919.

A. chromogenes (Gasperini, 1891; Kruse, 1896) Ford, 1927.

O. chromogenes Lehmann and Neumann, 1896.

A. cinereo-niger Lieske, 1921.

St. cinereonigeraromaticus (Berestnew, 1897) Neukirch, 1902.

A. citrea (Gasperini, 1894) Ford, 1927.

A. citroiremeus Nannizzi Pollacci, 1934.

A. cloacae Brussoff, 1919.

A. coccocidus Krassilnikov, 1955.

A. colorata Sanborn, 1926.

A. congolensis (Baerts, 1925) Brumpt, 1939.

A. convolutus (Chalmers and Christopherson, 1916) Brumpt, 1939.
Syn. *N. convoluta* Chalmers and Christopherson. According to Gonzalez Ochoa and Sandoval (1956), this is a synonym of *N. asteroides*.

A. coremiales Krassilnikov, 1949.

A. cretaceus (Krüger, 1905) Wollenweber, 1920, who considered it as a variety of *A. albus*.
Syn. *O. cretacea* Krüger, 1905.

A. cruoris (Macfie and Ingram, 1921) Brumpt, 1927.
Syn. *N. cruoris* Macfie and Ingram, 1921.

A. crystallophagus (Gray and Thornton, 1928) Bergey, 1930.

A. cuniculi (Schmorl) Gasperini, 1894.
Syn. *N. cuniculi* deMello and Fernandes, 1919.

St. cuniculi Foulerton and Price-Jones, 1901.

A. cylindraceus (deKorté, 1918) Brumpt, 1939.
Syn. *N. cylindracea* deKorté, 1918.

O. cylindracea Sartory, 1923.

A. dassonvillei (Brocq - Rousseu, 1907) Brumpt, 1939.
Syn. *N. dassonvillei* Liégard and Landrieu, 1911.

A. decussatus (Langeron and Chevallier, 1912) Brumpt, 1939.
Syn. *Discomyces decussatus* Langeron and Chevalier, 1912
N. decussata Castellani and Chalmers, 1913.
O. decussata Sartory, 1923.

A. denitrificans (Nikolaeva, 1914) Krassilnikov, 1949.

A. dermatonomus Bull, 1929 (*Polysepta dermatonomus* Thompson and Bisset, 1957).

A. dispar (Vidal, 1882) Brumpt, 1939.

A. donnae Dodge, 1935.

A. dori (deBeurmann and Gougerot, 1906) Brumpt, 1939.

O. doriae Sauvageau and Radais, 1892.

A. egypti Gohar *et al.*, 1954 (Ettlinger *et al.*, 1958).

A. elaeagni Roberg, 1934. Produces tubers on the roots of the oleander plant.

A. elephantis primigenii Omeliansky, 1909.

A. enteritidis (Pottien, 1902) Brumpt, 1927.
Syn. *N. enteritidis* Castellani and Chalmers, 1913.

St. eppingeri (Rossi-Doria, 1891) Namyslowsky, 1912.

A. equi (Chalmers and Christopherson, 1916) Brumpt, 1939.
Syn. *N. equi* (Dean, 1900) Chalmers and Christopherson, 1916.

A. erysipeloides (Neumann and Lehmann, 1895) Lachner-Sandoval, 1898.
Syn. *N. rosenbachi* (Gougerot, 1913).

St. erythrea Foulerton, 1902.

St. farcinica (Trevisan, 1889) Rossi-Doria, 1891.

A. ferrugineus (Naunyn) Krassilnikov, 1949.
Syn. *N. ferruginea* de Toni and Trevisan.

A. flava (Sanfelice, 1904) Ford, 1927.

A. flavus (Chester, 1901) Dodge, 1935.

A. fluorescens Krassilnikov, 1955.

St. Foersteri Cohn, 1875. Observed in concretions in the lachrymal canal. A facultative anaerobe, not pathogenic for laboratory animals. Numerous synonyms of this name are found in the literature. It is sufficient to list *Leptothrix oculorum* Sorokin, 1881; *Cl. foersteri* Winter, 1884; *Cl. dichotoma* Macé, 1888; *N. försteri* Trevisan, 1889; *O. försteri* Sauvageau et Radais, 1892; *St. försteri* Kruse, 1896; *N. aurea* Saint-Sévérin, 1902; *Cohnistreptothrix silberschmidti* Chalmers and Christopherson, 1916.

A. foulertoni (Chalmers and Christopherson, 1916) Brumpt, 1939.
Syn. *N. foulertoni* Chalmers and Christopherson, 1916.

St. freeri Musgrave and Clegg, 1907.
Syn. *A. freeri* Bergey, 1923.
Pr. freeri Krassilnikov, 1949. Definitely a *Nocardia*.

A. fusca Söhngen and Fol, 1914.
A. fuscus (Karwacki, 1911) Brumpt, 1939.
Syn. *N. fusca* Castellani and Chalmers, 1913.
A. gabritschevski (Berestnew, 1898) Krassilnikov, 1941.
A. garteni Brumpt, 1927.
Syn. *N. garteni* Gougerot, 1913.
A. gedanensis (Löhlein, 1909) Bergey, 1923.
A. gedanensis (Scheele and Petruschky, 1897) Ford, 1927.
Syn. *N. gedanensis* Chalmers and Christopherson, 1916.
St. gelatinosus Johan-Olsen, 1897.
A. genesii (Fróes, 1930) Dodge, 1935.
Syn. *N. genesii* Fróes, 1930.
A. gibsoni Dodge, 1935.
A. goensis (deMello and Fernandes, 1919) Dodge, 1935.
Syn. *N. goensis* deMello and Fernandes, 1919.
A. gonadiformis Bergey, 1930.
A. graminearus (Berestnew, 1897) Krassilnikov, 1949.
A. graminis (Bostroem, 1891) Topley and Wilson, 1929, 1946.
A. grisco-viridis Nikolaeva, 1914.
A. griseus variabilis Krassilnikov, 1949.
A. gruberi (Terni, 1894) Sanfelice, 1904.
Syn. *N. gruberi* Blanchard (In Bouchard, 1896).
A. guegueni (Brumpt, 1921) Brumpt, 1939.
Syn. *D. lingualis* Guegen, 1908.
N. lingualis Castellani and Chalmers, 1913.
A. guerrai (Langeron, 1929) Brumpt, 1939.
A. guignardi (Sauvageau and Radais, 1892) Ford, 1927.
A. gypsoides Henrici and Gardner, 1921.
A. halotricus ZoBell and Upham, 1944.
A. heimi Duché, 1934.
A. hobnesi (Gedoelst, 1902) Nannizzi Pollacci, 1934.
A. hofmanni (Gruber, 1891) Gasperini, 1894.
A. hominis Bostroem, 1890.
St. hominis II, III, IV (Foulerton, 1906 1910).
St. hominis (Berestnew, 1897).
Not *A. hominis* Waksman, 1919.
St. humifica Johan-Olsen, 1897.
A. incanescens Wollenweber, 1922.
A. indicus (Kanthack, 1893) Brumpt, 1939
Syn. *N. indica* var. *flava* Kanthack, 1893
A. innominatus Baldacci, 1939.
A. interproximalis (Fennel, 1918) Ford, 1927.
A. invulnerabilis (Acosta and Rossi, 1893; Kruse, 1896; Lachner-Sandoval, 1898) Ford, 1927. Isolated as laboratory contamination, and also from water. Withstands heating at 100–120°C. Grows in media containing 0.5 per cent copper sulfate, 0.5 per cent phenol, 1 per cent boric acid, and 0.01 per cent mercuric chloride.
St. israeli Kruse, 1896.
A. japonica (Petruschky, 1913) Ford, 1927.
A. japonicus (Aoyama and Miyamoto, 1901) Brumpt, 1939.
A. jollyi (Vuillemin, 1920) Brumpt, 1927.
A. keratolyticus (Acton and McGuire, 1931) Brumpt, 1939.
A. krainskii Duché, 1934.
A. krausei (Chester, 1901) Brumpt, 1927; Ford, 1927.
Syn. *N. krausei* Chalmers and Christopherson, 1916.
A. lacertae (Terni, 1896) Foulerton, 1912.
Syn. *A. sulfureus lacertae* Berestnew, 1897.
A. lanfranchii Sani, 1916.
Syn. *N. lanfranchii* deMello and Pais, 1918.
A. lasserei (Verdun, 1912) Brumpt, 1939.
Syn. *N. lasserei* (Verdun) Castellani and Chalmers, 1913.
St. lathridii (Petruschky, 1898) Ford, 1927.
A. leishmani (Chalmers and Christopherson, 1916) Brumpt, 1939.
A. lepromatis (deSouza-Araujo, 1929) Brumpt, 1939.
St. leucea Foulerton, 1902.
St. leucea saprophytica Foulerton, 1912.
A. levyi Dodge, 1935.

A. lieskei Duché, 1934.
Actinobacillus ligniersi (Brumpt, 1910) Brumpt, 1939.
A. liguire (Urizer, 1904) Nannizzi Pollacci, 1934.
A. lingualis (Weibel, 1888; non Gueguen, 1908) Brumpt, 1939.
A. liquefaciens (Hesse, 1892) Brumpt, 1939.
Syn. *N. liquefaciens* Castellani and Chalmers, 1913.
A. liquefaciens (Garten, 1895) Ford, 1927.
A. londinensis (Chalmers and Christopherson, 1916) Brumpt, 1939.
Syn. *N. londinensis* Chalmers and Christopherson, 1916.
A. longisporus Krassilnikov, 1941.
A. longisporus ruber Krassilnikov, 1941.
A. longisporus griseus Krassilnikov, 1941.
A. longissimus Krassilnikov, 1941.
A. luteolus (Foulerton and Jones, 1910) Brumpt, 1939.
A. luteo-roseus Gasperini, 1894.
A. macrodipodidarum (Fox, 1923) Dodge, 1935.
Syn. *N. macrodipodidara* Fox, 1923.
A. malenconi Duché, 1934.
A. matruchoti (Mendel, 1919) Brumpt, 1939.
A. melanogenes Rubentschik, 1928.
A. melanoroseus Issatschenko, 1927.
A. melanosporeus Krainsky, 1914.
St. melanotica Price-Jones, 1903.
A. metchnikovi (Sauvageau and Radais, 1892) Ford, 1927.
A. mexicanus (Boyd and Crutchfield, 1921) Brumpt, 1939.
Syn. *N. mexicana* Ota, 1928.
A. micetomae (Greco, 1916) Dodge, 1935.
Syn. *O. micetomae* Sartory, 1923.
A. mshagiensis Salimowskaja, 1928.
A. microflavus Krainsky, 1914.
A. mihi Caminiti, 1907.
A. mineaceus (Kruse, 1896) Lachner-Sandoval, 1898.
A. minimus (LeCalvé and Malherbe, 1900) Dodge, 1935.
A. minutissimus (Burchard) Brumpt, 1927.
Syn. *N. minutissima* (Verdun, 1912) Castellani and Chalmers, 1913.
A. mucosus Basu, 1943.
A. multifidus Krassilnikov, 1941.
A. muris-ratti (Schottmüller, 1914) Ford, 1927.
A. musculorum Hertwig, 1886.
A. mutabilis Masumoto, 1943; Gause *et al.*, 1957.
A. myricae Peklo, 1910.
St. necrophora Wilhelm, 1902.
A. necrophorus (Flügge, 1886) Lehmann and Neumann, 1926.
A. neddeni (Namyslowsky, 1912) Brumpt, 1939.
A. neschczadimenki (Chalmers and Christopherson, 1916) Dodge, 1935.
A. nicollei (Delanoë, 1928) Brumpt, 1939.
Syn. *N. nicollei* Delanoë, 1928.
A. niger (Rossi-Doria, 1891) Krassilnikov, 1949.
A. niger aromaticus Berestnew, 1897.
St. nigra (Rossi-Doria, 1891) Sanfelice, 1904.
A. nigricans Killian and Fehér, 1935.
A. nigrificans (Krüger, 1905) Wollenweber, 1920.
St. nigrescens Foulerton, 1901.
A. nitrogenes Sartory *et al.*, 1936.
A. nocardii (Foulerton, 1901) Buchanan, 1911.
A. nodosus (Beveridge, 1941) Hagan, 1943.
A. nondiastaticus Bergey, 1919. Grows at 65°C; differs from *S. thermodiastaticus* in not decomposing starch.
A. non-fluorescens Krassilnikov, 1955.
A. ochraceus Neukirch, 1902.
A. ochroleucus Neukirch, 1902. A culture described under same name was isolated from diseased potatoes by Wollenweber (1922) who considered it as a variety of *A. albus*.
A. odoratus Krassilnikov, 1941.
A. odorifer Koelz, 1936.
St. oidioformis Johan-Olsen, 1893.
A. oligocarbophilus (Beijerinck and Van Delden, 1903) Lantzsch, 1922.

St. orangica Berestnew, 1897.
A. orangico-niger Lieske, 1921.
A. orangicus (Rossi-Doria) Lieske, 1921.
A. panginensis (deMello and Fernandes 1919) Dodge, 1935.
Syn. *N. panginensis* deMello and Fernandes, 1919.
St. paulotrophus Beijerinck, 1914.
A. pelogenes Sawjalow, 1913.
A. penicilloides Sartory and Meyer, 1936.
A. phagocidus Krassilnikov, 1955.
A. phenotolerans Werkman and Patrick, 1932. Isolated from granuloma in man. Grows well in phenol-containing media. Gonzalez Ochoa and Sandoval (1956) considered it a synonym of *N. asteroides*.
A. pijperi (Castellani and Chalmers, 1919) Brumpt, 1939.
Syn. *N. pijperi* Castellani and Chalmers, 1919.
A. pinoyi (deMello and Fernandes, 1919) Dodge, 1935.
Syn. *N. pinoyi* deMello and Fernandes, 1919.
A. plurichromogenus (Caminiti, 1907) Dodge, 1935.
Syn. *N. plurichromogena* Caminiti, 1907.
A. pluricolor Gasperini, 1894.
Syn. *N. pluricolor* Terni, 1894; *N. pluricolor* deMello and Fernandes, 1919.
A. pluricolor diffundens (Berestnew, 1897) Lieske, 1921.
A. polychromogenus Dodge, 1935.
A. ponceti (Verdun, 1912) Brumpt, 1939.
Syn. *N. ponceti* (Verdun, 1912) Castellani and Chalmers, 1919.
A. pretorianus (Pijper and Pullinger, 1927) Brumpt, 1939.
Syn. *N. pretoriana* (Pijper and Pullinger, 1927).
A. protea (Schürmayer, 1900) Ford, 1927.
A. pseudonecrophorus Harris and Brown, 1927.
A. pseudotuberculosae (Flexner, 1898) Brumpt, 1939.
A. pseudotuberculosis (Keller) Dodge, 1935.
Syn. *N. pseudotuberculosis* deMello and Fernandes, 1919.
A. pseudotuberculosus (Flexner, 1898) Lehmann and Neumann, 1912.
A. pulmonalis Burnett, 1909; (Roger *et al.*, 1909) Brumpt, 1939.
A. puntonii Lopez Ortega, 1934.
A. purpurogenus Waksman and Curtis, 1916.
A. purpureus (Orloff, 1913) Brumpt, 1939.
St. putorii Dick and Tunnicliff, 1918.
A. putridogenes (Vezspremi, 1907) Nannizzi Pollacci, 1934.
A. putrificus Nikolaeva, 1914.
A. pyocyaneus Rullmann, 1895.
St. pyogenes Caminiti, 1907.
A. pyogenes (Chalmers and Christopherson, 1916) Dodge, 1935.
A. radiatus (Namyslowsky, 1912) Brumpt, 1939.
St. ratti Schottmüller, 1914.
A. ribeyroi Dodge, 1935.
A. rivierei (Verdun, 1912) Brumpt, 1939.
A. rodellae Dodge, 1935.
A. rogersii Brumpt, 1939.
Syn. *N. rogersi* deMello, 1919.
A. rosaceus Lieske, 1921.
O. rosella Krüger, 1905.
A. rosenbachi (Kruse, 1896) Holland, 1920.
A. roseolus Nadson, 1903.
O. rubea Wilbert, 1908.
St. rubea Chalmers and Christopherson, 1916.
A. ruber (Kruse, 1896) Sanfelice, 1904.
St. rubra (Kruse, 1896) Ford, 1927.
A. rubidaureus Lachner-Sandoval, 1898.
Syn. *A. mordoré* Thiry, 1897.
O. mordoré Sartory, 1923.
N. thiryei deMello and Pais, 1918.
A. sabrazes (Ferre and Faguet, 1895) Dodge, 1935.
A. saharae Killian and Fehér, 1935.
A. salvati Langeron, 1922; Fontoynont and Salvat, 1922.
A. sanfelicei (Redaelli, 1928) Nannizzi Pollacci, 1934.
Syn. *N. sanfelicei* Redaelli, 1928.
A. sanguinis Basu, 1937.

St. sanninii Ciferri, 1922.
A. saprophyticus Gasperini, 1892. Lieske, 1921.
A. saprophyticus var. *chromogenes* Gasperini, 1892.
A. sartoryi Dodge, 1935.
A. scabies var. *anglica* Baldacci and Spalla, 1956.
A. sendaiensis (Ping-Ting-Huang, 1933) Brumpt, 1939.
A. septicus MacNeal and Blevins, 1945.
A. serratus (Sartory *et al.*, 1930) Dodge, 1936.
A. silberschmidti (Chalmers and Christopherson, 1916) Dodge, 1936.
Syn. *N. silberschmidti* deMello and Fernandes, 1919.
A. somaliensis (Brumpt, 1906) St. John-Brooks, 1931.
A. sommeri (Greco, 1910) Brumpt, 1939.
A. spinae Velich, 1929.
A. spinosporus (Spini) Verlich, 1914.
St. spirilloides Johan-Olsen, 1893.
A. spitzi (Ligniéres and Spitz, 1904) Dodge, 1935.
Syn. *O. spitzi* Sartory, 1923.
A. splenicus (Gibson, 1930) Brumpt, 1939.
Syn. *N. splenica* Gibson, 1930.
A. spumalis (Sartory, 1923) Dodge, 1935. Appears to be a *Nocardia*.
St. taraxeri cepapi (Schottmüller, 1914) Ford, 1927.
A. tarozzii (Miescher, 1917) Dodge, 1935.
St. tartari Sanfelice, 1904.
O. tenax Krüger, 1905.
A. tenuis (Castellani, 1911) Dodge, 1935.
Syn. *N. tenuis* Castellani, 1911.
S. termitum Duché *et al.*, 1951.
A. thermotolerans Lieske, 1921.
A. thibiergei (Ravaut and Pinoy, 1909) Brumpt, 1939.
Syn. *N. thibiergei* Castellani and Chalmers, 1913.
A. thjottae (Thjøtta and Gundersen, 1925) Dodge, 1935.
A. thuillieri (deToni and Trevisan, 1889) Brumpt, 1939.
Syn. *N. thuillieri* Vuillemin, 1931.
A. tossicus (Rossi, 1905) Dodge, 1935.
A. totschidlowskii Serbinov, 1925.
A. toxicus Krassilnikov, 1955.
A. transvalensis (Pijper and Pullinger, 1927) Brumpt, 1939.
A. tricolor Wollenweber, 1922.
A. tyrosinaticus Beijerinck, 1914.
A. urethritidis (Rocek, 1920) Brumpt, 1939.
A. urinarius (Pijper, 1918) Brumpt, 1939.
A. valvulae (deMello and Pais, 1918) Nannizzi Pollacci, 1934.
Syn. *N. valvulae* deMello and Pais, 1918.
A. valvularis (Luginger, 1904) Ford, 1927.
A. valvulas destruens bovis Luginger, 1904.
A. verrucosus Nadson 1903; (Miescher, 1917) Brumpt, 1939.
A. violacea (Rossi-Doria, 1891) Ford, 1927.
A. waksmanii Bergey, 1930.
A. xanthostromus Wollenweber, 1922.
St. zopfi (Casagrandi) Caminiti, 1907.

The above is only a partial list of incompletely described cultures, most of which would now be included in the genus *Streptomyces*. Some cultures were listed as *Discomyces*, such as *D. pleuriticus* Vachetta (1882) and *D. pleuriticus canis familiaris* Rivolta. Various others were given under the generic names *Bacillus*, such as *B. actinoides* Smith, 1918; *Bacterium*, such as *B. actinocladothrix* Afanassiev, 1888; *Mycobacterium*, such as *M. paraffinicum* (Davis *et al.*, 1956), also under the anaerobic genus *Actinobacterium*, including *A. meyeri*, *A. abscensus*, *A. cellulitis* (Linhard, 1949); and as *Actinobacillus*, such as *Act. ligniersi* Brumpt, 1910.

A large number of cultures were simply given numbers, as done by Drechsler, Lieske, and many others. Finally, some were mentioned under a generic name, without even the affixation of a number, such as *Actinomyces* sp., *Streptomyces* sp., etc. All of these, with very few exceptions, need not be considered any further here.

Many of the above cultures have been isolated from excretions of man and labora-

tory animals. Some were found to be pathogenic to experimental animals. Most of them were not tested, however, for their pathogenicity.

Organisms Belonging to the Genus *Nocardia*

In addition to many of the forms listed previously, certain other incompletely described forms which probably belong to the genus *Nocardia* may be mentioned. These have been described under the generic names of *Nocardia* (N.), *Asteroides* (*As.*), and *Proactinomyces* (*Pr.*).

N. actinomyces Trevisan, 1889.
N. albida Chalmers and Christopherson, 1916.
N. albosporea Chalmers and Christopherson, 1916.
N. appendicis Chalmers and Christopherson, 1916.
Pr. aquosus Turfitt, 1944.
Pr. asteroides var. *crateriformis* Baldacci, 1937.
Pr. asteroides var. *decolor* Baldacci, 1937.
N. bifida (*B. bifidus* Tissier, 1901).
N. bovis Gougerot *et al.*, 1934.
N. cuniculi Snijders, 1924.
Pr. cyaneus (Beijerinck, 1914) Krassilnikov, 1941.
Pr. cyaneus antibioticus Gause, 1946.
N. erythropolis (Gray and Thornton, 1928) Waksman and Henrici, 1948.
N. filiformis (Boas, 1897) Vuillemin, 1931.
N. krainskii Chalmers and Christopherson, 1917.
N. lignieresi (Brumpt, 1910) Chalmers and Christopherson, 1916.
As. liskeyi Puntoni and Leonardi, 1935.
N. minima (Jensen, 1931) Waksman and Henrici, 1948.
N. pluricolor Namyslowsky, 1912.
As. pseudocarneus Puntoni and Leonardi, 1935.
Pr. pseudomadurae Baldacci, 1943.
N. ramosa (*B. ramosus* Veillon and Zuber, 1898).
Pr. restrictus Turfitt, 1944.
N. ripens (Eklund, 1883) Vuillemin, 1931.
N. saprophytica Chalmers and Christopherson, 1916.
N. sylvodorifera Castellani, 1911.

Various other specific names for organisms probably belonging to the genus *Nocardia* have been listed under several other genera, such as *Cladothrix* (*Cl. actinomyces* Rossi-Doria, 1891; Macé, 1897); *Cohnistreptothrix* (*Co. americana* Chalmers and Christopherson, 1916); *Discomyces* (*D. asteroides* Eppinger, 1891; Godoelst, 1902); *Flavobacterium* (*F. salmonicolor* den Dooren de Jong, 1927; Bergey, 1930); *Mycobacterium* (*M. albuvialum* Bergey, 1923); *Serratia* (*S. corralina* Hefferan, 1904; Bergey, 1923), and others. Krassilnikov (1949) listed or described numerous other forms belonging to this genus under the names of *Nocardia*, *Proactinomyces*, *Mycobacterium*, *Bacillus*, *Bacterium*, *Brevistreptothrix*, *Cladothrix*, *Cohnistreptothrix*, *Discomyces*, and various others. Thompson and Bisset (1957) suggested a new generic name *Polysepta*.

Descriptions Incomplete or Needed for Actinomycetes Producing Specific Antibiotics and Vitamins

Because of the growing importance of actinomycetes as producers of antibiotics and because of the desire to claim priority for a new antibiotic, many names have been introduced for species of *Streptomyces* and *Nocardia*. Often these names are mentioned, with incomplete descriptions or with no descriptions, in the patent literature or even in trade journals. An attempt was made to collect these. The list (Table 27) is far from complete, however.

A number of new species of actinomycetes belonging to the genus *Streptomyces* have been created by Gause *et al.* (1957–1959), who justified this by the fact that there was a great need for describing more organisms capable of producing antibiotics. Only a limited attempt was made in these descrip-

TABLE 27

Incompletely described antibiotic-producing species of Nocardia *and* Streptomyces

Organism	Reference	Antibiotic or vitamin
Nocardia		
N. acidophilus	J. Bacteriol. **54:** 281, 1947	Mycomycin
Pr. actinoides	Antibiotiki **2(5):** 44, 1957	Actinoidin
N. lurida	Antibiotics Ann. 1956–1957, 687, 693, 699	Ristocetin
N. narasinoensis	J. Antibiotics (Japan) **7A:** 1, 1954; **8B:** 253, 1955	Nocardorubin
Streptomyces		
S. albicans	Med. Parasitol. USSR 4, 1947	Actinolysin
S. albidofuscus	J. Antibiotics (Japan) **6A:** 140, 1953	Pyridomycin
S. albulus	Abstr. Papers 134th Ann. Meet. Am. Chem. Soc. Chicago, 1958, 22.	Antitumor substances
S. aminophilus	Antibiotics Ann. 1955–56, 236	Antibiotic 1968
S. arabicus	J. Antibiotics (Japan) **9B:** 62, 1956	Croceomycin
S. bacillaris	Folia Biol. **4:** 260, 1958	Various antibiotics
S. badius	J. Antibiotics (Japan) **3:** 582, 1949	Streptothricin
S. blastmyceticus	J. Antibiotics (Japan) **10A:** 40, 1957	Blastmycin
S. caiusiae	J. Sci. Ind. Res. India **16c:** 76–81, 1957	Antibiotic X
S. carcinomyceticus	Chemotherapy (Tokyo) **3:** 129, 1955	Carcinomycin
S. cellostaticus	Tohoku J. Exptl. Med. **67:** 173, 1958.	Cellostatin
S. chattanoogensis	Antibiotics & Chemotherapy **9:** 398, 1959	Tennecetin
S. chibaensis	J. Antibiotics (Japan) **11A:** 81, 1958	Cellocidin
S. chrestomyceticus	Giorn. Microbiol. **7:** 242, 1959	Aminocidin
S. cinnamonensis	Ann. Soc. Biol. Pernambuco **13:** 3, 1955; **14:** 9, 1956	Streptothricin-like antibiotic
A. circulatus var. *monomycini*	Antibiotiki **5(4):** 3, 1960.	Monomycin
A. coeruleus antibioticus	Antibiotiki **2(1):** 25, 1957	An antibiotic
S. colombiensis	U. S. 2,595,499, May 6, 1952	Vitamin B_{12}
S. chimensis	J. Antibiotics (Japan) **7B:** 168, 1954	Candimycin
S. fasciculatus	Antibiotics & Chemotherapy **3:** 718, 1953	Amicetin
S. flavofungini	Nature **181:** 908, 1958	Flavofungin
S. fluorescens	Folia Biol. **4:** 259, 1958	Fluorin
A. fluorescens	Antibiotiki **5(1):** 25, 1960	Actinomycin
A. fradiae var. *spiralis*	Antibiotiki **1:** 4, 1956	Colimycin; probably neomycin
S. ganmycicus	J. Antibiotics (Japan) **9A:** 8, 113, 1956; **9B:** 160, 1956	Ganmycin + Carcinomycin
S. ganmyceticus	J. Antibiotics (Japan) **9A:** 6, 9, 113, 1956	Carzinocidin
S. globisporus tundramycini	Bull. Moscow Soc. Nat. Sci. Biol. **62(2):** 79, 1957.	Tundramycin
S. graminofaciens	Antibiotics & Chemotherapy **3:** 1283, 1953; Antibiotics Ann. 1953–1954, 171	Streptogramin
S. griscoplanus	Antibiotics Ann. 1956–1957, 730	Alazopeptin
S. grisinus	Folia Biol. **4:** 263, 1958	Grisin
S. griseus var. *farinosus*	Bacteriol. Proc. p. 18, 1954	Streptolin
S. griseus var. *spiralis*	Antibiotics Ann. 1959–1960, 194.	Aspartocin
S. hepaticus	Brit. Pat. 730,341, May 18, 1955	

TABLE 27—*Continued*

Organism	Reference	Antibiotic
A. jucosus	Krassilnikov, 1955	Biomycin
S. levoris	Folia Biol. **4:** 260, 1958; resembles *S. griseus* var. *candicidinus*	Levorin
S. leydenematis	Trans. Am. Microscop. Soc. **5:** 376, 1953.	
S. lilacinus	J. Antibiotics (Japan) **9B:** 81, 1956	Cladomycein
S. longisporus	Folia Biol. **4:** 263, 1958	Longisporin
S. luteochromogenes	J. Antibiotics (Japan) **6A:** 183, 1953	Phthiomycin
S. luteolutescens	Kurilowicz in Udientzev *et al.*, p. 151, 1959	Antitumor agent
S. mediterranei	Antibiotics Ann. 1959–1960, 262.	Rifomycin
S. melanochromogenes	Kurilowicz in Udientzev *et al.*, 1959	Antitumor agent
S. melanosporus (*melanosporofaciens*)	Giorn. Microbiol. **7:** 207, 1959	Melanosporin and Elaiophylin
S. natalensis	Antibiotics Ann. 1957–1958, 878	Pimaricin
S. orchidaceus	Antibiotics & Chemotherapy **5:** 204, 1955; British Patent 768,007, February 13, 1957	Cycloserine
S. paucisporogenes	Ann. Pharm. Franc. **16:** 585, 1958	?
S. phaeofaciens	Japan. Med. J. **5:** 327, 1952	Phaeofacin
S. phoenix	Antibiotics & Chemotherapy **3:** 788, 1953	Rhodocidin
S. pleofaciens	Antibiotics Ann. 1954–1955, 806	Pleomycin
S. plicatus	Brit. Pat. Spec. 707,332, Apr. 14, 1954	Antibiotics C and D
S. pluricolorescens	J. Antibiotics (Japan) **9A:** 75, 1956	Pluramycin
S. primycini	Pharmazie **11:** 304, 1956	?
S. racemochromogenus	J. Antibiotics (Japan) **9B:** 170, 1956; **11B:** 277, 1958.	Racemomycin
S. raffinosus	Folia Biol. **4:** 259, 1958	?
S. recifei	(Lima *et al.*) Morais, Lima and Maia, Syn. *N. recifei* Lima *et al.* (An. Soc. Biol. Pernambuco **15:** 239, 1957)	?
S. rutgersensis var. *casteranse*	Rev. Inv. Agr. Buenos Aires **8:** 263, 1954	Camphomycin
S. sakaiensis	Ann. Rept. Takeda Research Lab. **14:** 8, 1955	Monilin
S. salmonicida	J. Bacteriol. **58:** 659, 1949	?
S. sindenensis	Ann. Rept. Takeda Research Lab. **13:** 41, 1954	Allomycin
S. subtropicus	Brit. Med. J. Nov. 12, 1955, Doklady Akad. Nauk. SSSR **99:** 827, 1954	Albomycin (grisein)
S. toxicus	Folia Biol. **4:** 259, 1958	Necrotin
S. toyocaensis	Japanese Pat. 32-3049, 1957	Toyocamycin
S. vendargus	Australian Pat. 3985, Oct. 20, 1954	Oxytetracycline and Vengicide
S. verticillus	J. Antibiotics (Japan) **12A:** 285, 1959	Pleomycin
S. vinaceus-drappus	Brit. Pat. Spec. 708,686, May 5, 1954	Amicetin
A. violaceus-cristallomicini	Antibiotiki **2(5):** 58, 1957	Crystallomycin
S. viridifaciens	U.S. 2,712,517, July 5, 1955; Brit. Pat. Spec. 770,065, Mar. 13, 1957	Tetracycline and/or chlortetracycline
S. viridosporus	Brit. Pat. Spec. 712,547, July 28, 1954	Sistomycosin
S. vulgaris	Folia Biol. **4:** 260, 1958	Pneumocin
S. xanthochromogenes	Bull. Agr. Chem. Soc. Japan **30:** 469, 1956	Xanthicin
S. zaomyceticus	J. Antibiotics (Japan) **7A:** 134, 1954	Zaomycin

TABLE 28

Incompletely described Streptomyces *species of Gause et al.* (1957)

A. abikoensum var. *spiralis*
A. acrimycini
A. acrimycini var. *globosus*
A. albidus var. *invertens*
A. alborubidus
A. albovinaceus
A. atroolivaceus
A. aurentiogriseus
A. aurini
A. badius
A. bicolor
A. biverticillatus
A. candidus var. *alboroseus*
A. chromofuscus
A. cinnabarinus
A. cinnamonensis var. *proteolyticus*
A. coelicolor var. *achrous*
A. coelicolor var. *flavus*
A. coeruleofuscus
A. coeruleorubidus
A. coerulescens
A. coerulescens var. *longisporus*
A. cremeus
A. cyanofuscatus
A. daghestanicus
A. flaveolus var. *rectus*
A. flavotricini
A. flavidovirens
A. flavidovirens var. *fuscus*
A. fradiae var. *spiralis*
A. fumanus
A. glaucescens
A. glaucescens var. *badius*
A. globisporus var. *caucasicus*
A. globisporus var. *flavofuscus*
A. gobitricini
A. griseoloalbus
A. griseoincarnatus
A. griseomycini
A. griseorubens
A. griseoruber
A. griseorubiginosus
A. griseorubiginosus var. *spiralis*
A. griseostramineus
A. iverini
A. kurssanovii
A. lateritius
A. litmocidini
A. malachiticus
A. mutabilis
A. nigrescens
A. olivaceoviridis

TABLE 28—*Continued*

A. prunicolor
A. roseofulvus
A. roseolilacinus
A. roseolus
A. roseoviolaceus
A. roseoviridis
A. rubiginosohelvolus
A. rubiginosus
A. syringini
A. toxytricini
A. umbrinus
A. variabilis
A. variabilis var. *roseolus*
A. venezuelae var. *spiralis*
A. violaceorectus
A. violaceus var. *rubescens*
A. violascens
A. viridoviolaceus

tions of "new" organisms to emphasize the ability of the cultures to produce melanin pigments, an important and characteristic property for distinguishing and identifying species of *Streptomyces*, as brought out in the various editions of Bergey's Manual. In spite of the fact that these descriptions of "new" organisms had been undertaken for the purpose of identifying antibiotic-producing cultures, no attempt was made to list any antibiotic for the various "new" species; all one finds are such expressions as: "strong repression," "weak activity," "no activity." Even the antibiotic "albomycin" that has been greatly publicized by the senior author is not listed in this treatise; neither is the species *A. subtropicus*, which produces this antibiotic, described. Very little effort has been made in creating these species to compare the newly isolated cultures with those previously described and well known in the literature. Many of the new descriptions remind one of known organisms.

For these reasons, this collection of "new" species is listed here (Table 28) as incompletely described, or at least as requiring further information for proper identification.

Appendices

Two appendices are given here, each of which is of special significance.

I. Since the colors of the substrate growth, the aerial mycelium, and the spores, as well as the pigment dissolved in the medium, are highly important in classifying actinomycetes, a well-recognized and universally available standard must be used for color evaluation. Unfortunately, some of the best standard charts and volumes are not generally accessible. Hence, a simplified system, readily understood, must be used. Such a system has been proposed by Lindenbein (1952). It is presented here, with certain modifications and in terms of English equivalents, as Appendix I. Several color charts are available for this purpose. The following standards may be consulted: Ridgeway (1912), Sacchardo (1912), Maerz and Paul (1930), and Munsell.

II. A knowledge of the chemical composition of the media used for the growth of actinomycetes is essential for characterization of species and varieties. Suitable media also are essential for good sporulation and for the production of important biochemical products, notably antibiotics and vitamins. Numerous media have been proposed for the growth of actinomycetes, to serve one purpose or another. To list them all here is hardly necessary. Only a few have been selected. These include synthetic, artificial organic, and complex natural media.

For microscopic examination of the culture, it is desirable to make stained preparations. Some of the general principles underlying light microscopic and electron-microscopic preparations have been discussed in Chapter 2 of Volume I.

Appendix I

Color Designations for Describing Actinomycetes (Lindenbein)

1. White: (a) snow-white (*niveus*), (b) glossy white (*candidus*), (c) silver-white (*argenteus*), (d) milk-white (*lacteus*), (e) chalk-white (*cretaceus*), (f) gray-white (*farinaceus*); also cream, egg-shell, and ivory.

2. Violet: (a) bluish-violet (*violaceus*), (b) reddish-violet (*lilaceus*), also lavender, mauve, purple.

3. Blue: (a) dark blue (*caeruleus*), (b) cornflower blue (*cyaneus*), (c) sky blue (*azureus*), (d) gray-blue (*caesius*), (e) yellowish-blue (*lividus*).

4. Green: (a) grass-green (*viridis*), (b) emerald green (*smaragdinus*), (c) blue-green (*glaucus*), (d) forest-green (*prasinus*), (e) olive-green (*olivaceus*).

5. Yellow: (a) light yellow (*flavus*), (b) deep yellow (*luteus*), (c) citron-yellow (*citrinus*), (d) golden yellow (*aureus*), (e) sulfur-yellow (*sulfureus*), (f) white-yellow (*stramineus*), (g) brownish-yellow (*gilvus*), (h) egg-yolk yellow (*vitellinus*), (i) pale yellow (*luridus*), (k) greenish-yellow (*galbus*).

6. Orange: (a) light orange (*aurantiacus*), (b) dark reddish-orange (*croceus*).

7. Red: (a) dark red (*ruber*), (b) carmine-red (*puniceus*), (c) scarlet red (*coccineus*), (d) fire-red (*igneus*), (e) pale carmine (*roseus*), (f) flesh-red (*carneus, incarnatus*), (g) purple-red (*purpureus*), (h) cinnabar-red (*cinnabarinus*), (i) lead-red (*miniatus*), (k) brick-red (*lateritius*), (l) blood-red (*sanguineus*), (m) brownish-copper red (*cupreus*), (n) light yellow-red (*rutilus*); also pink, coral-pink, rose, and wine-colored (*vinaceus*).

8. Brown: (a) light brown (*brunneus*),

(b) dark brown (*umbrinus*) (c) chestnut brown (*badius*), (d) reddish-brown (*fuscus*), (e) yellow-rusty-brown (*ferrugineus*), (f) greenish-brown (*hepaticus*), (g) cinnamon-brown (*cinnamomeus*); also beige, tan, and ocher.

9. Gray: (a) greenish-gray (*griseus*), (b) ash-gray (*cinereus*), (c) white-gray (*incanus*), (d) brownish-gray (*fumigatus*), (e) reddish-gray (*murinus*), (f) bluish-gray (*plumbeus*).

10. Black: (a) gray-black (*niger*), (b) coal black (*ater*), (c) brownish-pitch-black (*piceus*), (d) greenish-jet-black (*coracinus*), (e) blue-black (*atramentarius*).

Appendix II

Certain Important Media for the Study of Actinomycetes

Krainsky (1914) and Waksman and Curtis (1916) were the first to report on the significance of simple synthetic media in the study of the morphological and cultural properties of actinomycetes. Conn (1921) made a careful comparison of the growth of 75 cultures of actinomycetes on a large number of media. He came to the conclusion that "extreme variation in chromogenesis is possible with some of the cultures studied, according to the composition of the medium, and some cultures even vary greatly when studied at different times on the same medium. The appearance of one of these organisms on any medium should not be described until it has been cultivated on several lots of this medium at different times. No culture, moreover, can be considered nonchromogenic until it has been studied on a great variety of different protein-free media."

The following have been selected as representing the more important media recommended for the study of actinomycetes. All constituents are reported in grams per liter.

1. Sucrose nitrate agar:

Sucrose	30.0 gm
$NaNO_3$	2.0 gm
K_2HPO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
KCl	0.5 gm
$FeSO_4$	0.01 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 7.0 to 7.3

This medium is frequently also known as "Czapek's agar," or as "Czapek's solution agar." Glycerol or glucose may besubstituted for sucrose, giving glycerol-, or glucose-nitrate agar. Ammonium chloride may be substituted for $NaNO_3$, giving sucrose-ammonium agar.

2. Glucose-asparagine agar:

Glucose	10.0 gm
Asparagine	0.5 gm
K_2HPO_4	0.5 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 6.8

Meat extract (2.0 gm) may be added to this medium. Tap water may be used.

3. Glycerol-asparagine agar:

Glycerol	10.0 gm
Asparagine	1.0 gm
K_2HPO_4	1.0 gm
Agar	20.0 gm
Tap water	1000.0 ml

Adjust to pH 7.0 with NaOH.

4. Glycerol-asparaginate agar I:

Glycerol	35.0 gm
Ammonium lactate	6.5 gm
Sodium asparaginate	3.5 gm
K_2HPO_4	2.5 gm
NaCl	5.0 gm
$CaCl_2$	0.1 gm
$MgSO_4$	0.3 gm
Agar	20.0 gm
Distilled water	1000.0 ml

This medium is often spoken of as Ushinsky's, especially when used as a solution, without agar.

5. Glycerol-asparaginate agar II:

Glycerol	10.0 gm
Sodium asparaginate	1.0 gm

K_2HPO_4	1.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml

This medium is often known as Conn's.

6. Glycerol-glycine agar:

Glycerol	20.0 gm
Glycine	2.0 gm
K_2HPO_4	1.0 gm
NaCl	2.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
$FeSO_4$	0.1 gm
$CaCO_3$	0.2 gm
Agar	18.0 gm
Distilled water	1000.0 ml

Adjust to pH 7.2 with NaOH

This medium is often known as Plotho's.

7. Glycerol-calcium malate agar:

Glycerol	10.0 gm
Calcium malate	10.0 gm
NH_4Cl	0.5 gm
K_2HPO_4	0.5 gm
Agar	15.0 gm
Distilled water	1000.0 ml

Glucose or mannitol may be used (20 gm) to replace the glycerol. Calcium citrate may be used to replace the malate.

8. Glucose-ammonium salt agar:

Glucose	10.0 gm
$(NH_4)_2HPO_4$	4.0 gm
NaCl	5.0 gm
K_2HPO_4	2.0 gm
$MgSO_4 \cdot 7H_2O$	1.0 gm
$CaCl_2$	0.4 gm
$FeSO_4 \cdot 7H_2O$	0.02 gm
$MnSO_4 \cdot 7H_2O$	0.01 gm
Agar	15.0 gm
Distilled water	1000.0 ml

9. Glycerol-ammonium salt agar:

Glycerol	10.0 gm
NH_4Cl	1.0 gm
K_2HPO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
NaCl	0.5 gm
$CaCO_3$	1.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 7.0

The ammonium salt may be replaced by KNO_3. This medium was proposed by Masumoto (1952).

10. Glycerol-urea agar:

Glycerol	15.0 gm
Urea	2.0 gm
K_2HPO_4	0.5 gm
$MgSO_4$	0.5 gm
NaCl	0.5 gm
$FeSO_4$	0.1 gm
Agar	15.0 gm
Distilled water	1000.0 ml

11. Glucose-tyrosine agar:

Glucose	10.0 gm
Tyrosine	1.0 gm
$(NH_4)_2SO_4$	0.5 gm
K_2HPO_4	0.5 gm
Agar	15.0 gm
Distilled water	

Reaction made neutral with NaOH.

12. Glucose-peptone agar:

Glucose, crude	40 gm
Peptone	10 gm
Agar, powdered	20 gm
Distilled water	1000.0 ml

pH 5.6

Dissolve glucose and peptone in 500 ml distilled water. Dissolve agar, by heating at 100°C, in another 500 ml water. Mix the two solutions, cool, add 10 gm egg-albumin dissolved in 50 ml water. Shake, steam for 30 min, allow clot to settle, filter, distribute in tubes, and autoclave at 115°C for 10 min. This medium, also known as Sabouraud's agar, is fairly acid.

13. Tyrosine-casein-nitrate agar:

Tyrosine	1.0 gm
Sodium caseinate	25.0 gm
Sodium nitrate	10.0 gm
Agar	15.0 gm
Tap water	1000.0 ml

This medium has been recommended (Menzies and Dade, 1959) for the isolation of *Streptomyces scabies* from potato scab lesions

or from soil. It inhibits spreading bacteria and favors the production of a dark brown pigment closely encircling colonies of the pathogen. Since almost all pathogenic isolates of *S. scabies* produce a dark pigment, the selection of the probable pathogen from dilution plates containing other actinomycetes is favored.

14. Peptone-beef extract or nutrient agar:

Peptone	5.0 gm
Beef extract	5.0 gm
NaCl	5.0 gm
Agar	15 to 20 gm
Distilled water	1000.0 ml
pH 7.2 to 7.4	

Frequently 10 gm of peptone is used. Glucose (10 gm) or glycerol (15 gm) may be added to give glucose- or glycerol-peptone agar. Tap water is often used in place of distilled water. In a liquid state without agar, these media are designated as nutrient broth, glucose-peptone broth, or glycerol-peptone broth, respectively.

15. Peptone-beef-salt agar:

Glucose	10.0 gm
Peptone	5.0 gm
Beef extract	5.0 gm
K_2HPO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
KCl	0.5 gm
Agar	15.0 gm
Distilled water	1000.0 ml

The organic nutrients may be reduced to one half or even one tenth of above concentration, in order to favor production of aerial mycelium in some cultures of actinomycetes.

16. Glycerol-peptone-beef agar:

Glycerol	20.0 gm
Peptone	5.0 gm
Beef extract	3.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml
pH 7.0	

17. Tryptone-yeast agar:

Tryptone	1.0 gm
Yeast extract	1.0 gm
NaCl	8.5 gm
Agar	17.0 gm
Tap water	1000.0 ml

18. Glucose-yeast-ammonium agar:

Glucose	10.0 gm
Yeast extract	1.0 gm
$(NH_4)_2HPO_4$	1.0 gm
KCl	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.2 gm
Agar	15.0 gm
Distilled water	1000.0 ml
pH 7.0	

19. Gelatin media:

Gelatin	160–200 gm
Tap water	1000.0 ml
Adjust to pH 7.4	
Sterilize 30 minutes at 110°C	

This medium may be supplemented with glucose (20.0 gm) and peptone (5.0 gm) to give glucose-peptone gelatin.

20. Gelatin agar medium:

Peptone	5.0 gm
Beef extract	3.0 gm
Gelatin	4.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml
pH 7.0	

21. Starch agar:

Soluble starch	10.0 gm
$NaNO_3$	1.0 gm
K_2HPO_4	0.3 gm
NaCl	0.5 gm
$MgCO_3$	1.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml

$CaCO_3$ (3 gm) and $MgSO_4 \cdot 7H_2O$ (1 gm) may replace the $MgCO_3$. $(NH_4)_2SO_4$ (2 gm) or asparagine (0.05 gm) may be used to replace the nitrate.

22. Starch-casein agar:

Soluble starch	10.0 gm
Casein (dissolved in NaOH)	1.0 gm
K_2HPO_4	0.5 gm
Agar	15.0 gm
Water	1000.0 ml
pH 7.0 to 7.5	

23. Starch-peptone-beef-agar:

Starch	10.0 gm
Peptone	5.0 gm
Meat extract	3.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 7.0

24. Glycerol-starch-glutamate agar:

Glycerol	10.0 gm
Starch	10.0 gm
Sodium glutamate	1.0 gm
$NaNO_3$	0.5 gm
Proline	0.25 gm
Vitamin B	0.01 gm
K_2HPO_4	0.25 gm
$MgSO_4$	0.25 gm
$FeSO_4$	0.01 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 7.0 to 7.2

Shinobu (1958b) considered this one of the best synthetic media for growth of actinomycetes, especially for production of aerial mycelium.

25. Egg-albumin agar:

Glucose	10.0 gm
Egg albumin	0.15 gm
K_2HPO_4	0.5 gm
$MgSO_4 \cdot 7H_2O$	0.2 gm
$Fe_2(SO_4)_3$	Trace
Agar	15.0 gm
Distilled water	1000.0 ml

Egg albumin is first dissolved in water and made neutral to phenolphthalein with N/10 NaOH.

26. Potato-glucose agar:

Peeled potatoes	200.0 gm
Glucose	20.0 gm
$CaCO_3$	0.2 gm
$MgSO_4 \cdot 7H_2O$	0.2 gm
Agar	15.0 gm
Tap or distilled water	1000.0 ml

pH 6.8 to 7.2

27. Potato-peptone-glycerol agar:

Potato	100.0 gm
Peptone	2.0 gm
Glycerol	5.0 gm
$MgSO_4$	0.5 gm
K_2HPO_4	0.5 gm
NaCl	0.5 gm
$FeSO_4$	0.01 gm
Agar	15.0 gm
Tap water	1000.0 ml

The potatoes are cut into small cubes to which 100 ml of water is added and the whole steamed for three quarters of an hour. The extract is strained through fine muslin without squeezing the pulp. The other nutrients are dissolved in 500 ml of water which is then added to the potato extract, and the whole steamed for three quarters of an hour. The mixture is then made up to bulk, standardized and filtered, after which the agar is added.

28. Glucose-yeast extract-beef-peptone agar:

Glucose	10.0 gm
Yeast extract	10.0 gm
Beef extract	4.0 gm
Peptone	4.0 gm
NaCl	2.5 gm
Distilled water	1000.0 ml

This medium is often known as Emerson's. Various modifications of this medium are used. The concentration of the first two constituents may be reduced to 1.0 gm per liter; the NaCl may be left out.

29. Glucose-yeast extract agar:

Glucose	10.0 gm
Yeast extract	10.0 gm
Agar	15.0 gm
Tap water	1000.0 ml

pH 6.8

This medium may also contain 0.5 gm K_2HPO_4.

30. Glucose-casein digest-yeast-beef agar:

Glucose	10.0 gm
Yeast extract	1.0 gm
Beef extract	1.0 gm
N-Z-Amine A	2.0 gm
Agar	15.0 gm
Distilled water	1000.0 ml

pH 7.3

This medium is usually known as Bennett's agar.

31. Glucose-yeast-malt agar:

Glucose	4.0 gm
Yeast extract	4.0 gm
Malt extract	10.0 gm
Agar	20.0 gm
Distilled water	1000.0 ml
pH 7.3	

32. Dextrin-casein digest agar:

Dextrin	10.0 gm
Yeast extract	1.0 gm
Beef extract	1.0 gm
Casein digest (N-Z-Amine A)	2.0 gm
$CoCl_2 \cdot 7H_2O$	0.02 gm
Agar	20.0 gm
Distilled water	1000.0 ml
Adjust to pH 7.3	

This medium is often known as Hickey and Tresner's agar.

34. Oatmeal agar:

Rolled oats	20 to 65 gm
Tap water	1000.0 ml

Cook to thin gruel in double boiler, filter through several layers of cheesecloth, and make up to a liter while still hot. Add 18 to 20 gm agar. Adjust to pH 7.2 with NaOH.

34. Tomato paste-oatmeal agar:

Heinz baby oatmeal food	20.0 gm
Tomato paste	20.0 gm
Tap water	500.0 ml

Add these two ingredients to the 500 ml of boiling tap water. Do not adjust pH.

Difco agar	15.0 gm
Tap water	500.0 ml

Melt by steaming at 100°C for 15 to 20 min. Do not adjust pH. Mix the two solutions, steam at 100°C for 10 min, dispense, and sterilize for 15 min at 121°C.

35. Soil extract agar:

Beef extract	3.0 gm
Peptone	5.0 gm
Agar	15.0 gm
Soil extract	1000.0 ml
pH 7.0	

The soil extract is prepared by treating 1 kg garden soil with 2.5 liters of tap water for 1 hour in autoclave at 15 lb pressure. Filter hot. Add talc for clarification if necessary.

36. Carbon nutrition medium:

Carbon source	10.0 gm
$(NH_4)_2SO_4$	2.64 gm
KH_2PO_4	2.38 gm
K_2HPO_4	5.65 gm
$MgSO_4 \cdot 7H_2O$	1.00 gm
$CuSO_4 \cdot 5H_2O$	0.0064 gm
$FeSO_4 \cdot 7H_2O$	0.001 gm
$MnCl_2 \cdot 4H_2O$	0.0079 gm
$ZnSO_4 \cdot 7H_2O$	0.0015 gm
Agar	15.0 gm
Distilled water	1000.0 ml

Some carbon compounds may have to be sterilized separately either by filtration or by heating in aqueous solution. This medium was used by Pridham and Gottlieb (1948), and has found general application in the study of utilization by actinomycetes of different carbon sources (Fig. 66).

37. Medium for nitrate reduction:

Peptone	5.0 gm
Meat extract	3.0 gm
KNO_3	1.0 gm
Distilled water	1000.0 ml
pH 7.0	

38. Medium for hydrogen sulfide production:

Peptone-iron agar (Difco)	36.0 gm
Yeast extract	1.0 gm
Distilled water	1000.0 ml

39. Cellulose medium:

Filter paper saturated with synthetic solution, free from other carbon sources.

40. Potato plug:

Plugs of potatoes of desirable size and shape; distilled water added and sterilized.

The importance of the variety and health of potato has been discussed by Grein and Küster (1955).

41. Milk or litmus milk:

Skim milk powder is used. Litmus or brom-cresol may be added.

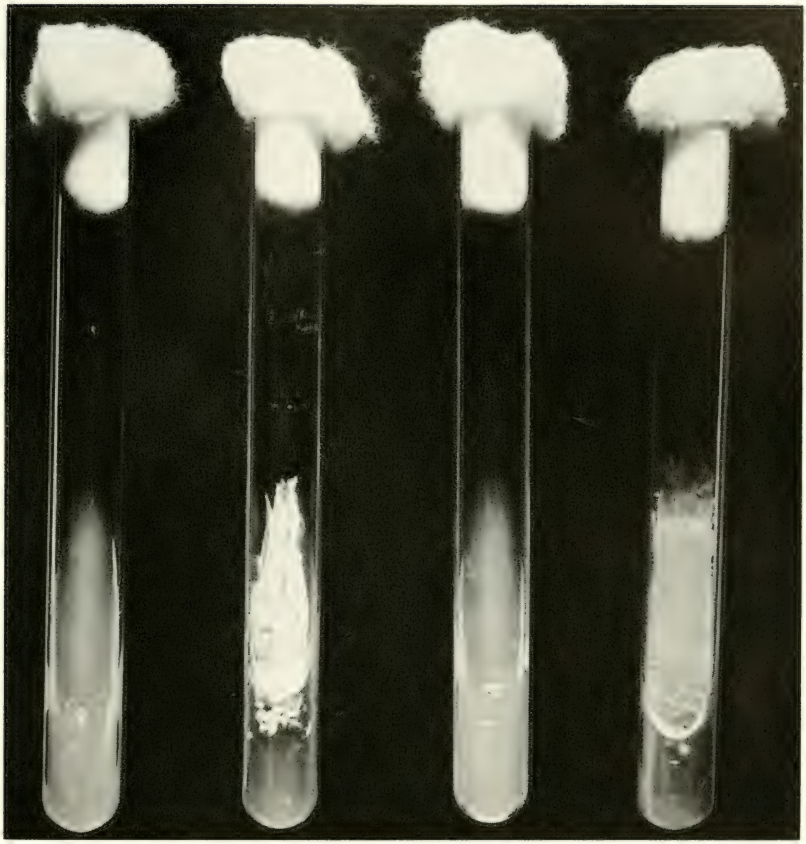

FIGURE 66. Growth responses of streptomycetes, on chemically defined medium, to various carbon sources (Courtesy of T. G. Pridham).

42. Melanin formation medium:

Yeast extract	1.0 gm
L-Tyrosine	1.0 gm
NaCl	8.5 gm
Agar	16.0 gm
Tap water	1000.0 ml

Use 4 to 5 ml portions in test tubes. Incubate at 27°C and read after 1 to 2 days and after 4 days.

43. Other media:

A variety of other media are frequently used, often depending upon the nature of the

organism and the problem. This is true particularly of the pathogenic forms, of organisms not growing readily upon ordinary media, and of those used for special purposes, such as antibiotic production. Among the complex media may be listed certain egg media (Dorset's), blood agar, blood serum, carrot plug, and others (Waksman, 1919; Levine and Schoenlein, 1930; Pridham *et al.*, 1956).

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INDEX OF ORGANISMS

Boldface numerals indicate description of organisms.

GENERAL INDEX